

Poor outlook on privacy protection

The British Government is persisting with a second-rate bill to regulate data banks. Can it be persuaded to think again?

AT birthday parties in the United States, it is now fashionable for people devoted to liberal causes such as racial non-discrimination to be given by their friends a copy of the record of their activities accumulated over the years by the Federal Bureau of Investigation. This has been made possible by the Freedom of Information Act, one of the steps taken during the Carter Administration to safeguard the rights of individuals against the misuse by governments of techniques of data gathering and manipulation, old as well as new. On present form, it will be some time before this quaint custom can be taken up elsewhere. Although most governments of Western Europe are signatories of the Council of Europe's convention on data protection, designed to require that data banks containing personal information about individuals should not be misused, the convention allows governments specifically to exclude from the provision that individuals should have access to records concerning them those data files judged to bear on national security and the prevention of crime. And while this derogation of the convention is permissive only, the signs are that many governments will take advantage of it. Certainly that is what the British Government intends.

The government, like many others in Western Europe, is in a cleft stick. The European convention has come into being primarily to ensure that data banks should not be used transnationally in ways that compromise people's civil rights. Thus the convention prohibits the transfer of data from an adherent country to one not party to the convention. Clearly this restriction would seriously impede the proper activities of all kinds of businesses, transnational companies and computer bureaux in particular, so that the British Government has for some time been under pressure to comply with the convention. The bill intended for this purpose, introduced into the previous parliament and drawn as the bare minimum consistent with ratification, was a procedural casualty of the general election in June. An amended version of the bill, more clearly drafted than its predecessor but otherwise not very different, was launched in the House of Lords in July, will reappear in an amended form when Parliament reassembles next month and will then run the gauntlet of the House of Commons some time next year. Sooner or later, there will be an inadequate statute on the books.

Registrar

That the British Government should be pilloried on this account is ironical, for the European convention derives largely from the principles put forward by the Younger Committee in 1972. Briefly, the convention requires that computerized data banks should be lawfully and "fairly" compiled for purposes specified in advance, that individuals should be entitled to know which data banks contain information about them and to correct it should it be false and that there should be exemptions in the interests of national security, public safety and "the monetary interests of the state and the suppression of criminal offences". The British Government's plan is that there should be a public official called a registrar whose chief function will be to license the compilation of data banks and the uses thereafter made of them, and a tribunal to which the managers of data banks may appeal. One of the complaints is that the official will not be an independent ombudsman but rather a creature of the government department (the Home Office) responsible for administering the law. Another is that the

government has added to the list of exemptions allowed by the convention "the control of immigration". A third is that the bill says nothing about the special needs of privacy in the uses made of medical records. Finally, there is a general suspicion that the bill will be an administrative mess.

Quite apart from the needs of the computer industry, it is only proper to acknowledge that even this unsatisfactory bill will be better than nothing. The requirement that the existence of most data banks, even many maintained by government departments, will become part of the public record will be a big step. So will be the restrictions on the transfer of data collected for one purpose to those who would use it for some other purpose. But the central stumbling block is what it has always been — the danger that the proposed law will institutionalize undesirable practices. Thus Clause 27 allows government data banks to be exempted in the interests of "safeguarding national security", in which case it will suffice that any member of the cabinet should declare them as such. Clause 28 goes further, exempting from restrictions transfers of data that will help in the detection of crime, the prosecution of offenders, the collection of tax and the control of immigration. And individuals will not have the right to inspect entries referring to them in records compiled for those purposes.

Security

These exceptions are too broadly drawn. National security, acknowledged by the European convention to be a proper cause for exemption, is nevertheless too insidious an excuse to be left for definition to individual ministers, any one of whom might choose to identify it with the parochial needs of his own department (to keep non-secrets secret, for example). So at the least there is a need for a closer definition of the concept. Ideally, there should be some mechanism by which the existence of such files were registered with an official charged with the administration of the act but required to keep secrecy on the government's legitimately secret files. The proposed exemptions for the "control of immigration" are similarly objectionable.

But why should data banks compiled for the prosecution of crime in any case be closed to inspection by those whose names are included? The usual argument is that allowing people to be warned in advance of evidence held against them would increase the difficulties of mounting successful prosecutions. The argument could well be false. As things are, police evidence is a hotchpotch of fact and hearsay, much of which is bound to be inaccurate. Knowing whom to charge with a specific crime is often complicated by the mass of misinformation about innocent "suspects" that has accumulated. And accurate information will not cease to be good evidence once the person involved knows what it is.

The row about medical records is, however, more urgent. The plan is that confidentiality may be overridden in the interests of law and order, tax collection and the control of immigration. The bill says that the managers of data banks will be free to pass on data whenever confidentiality would "prejudice any of these matters". What this implies is that the police or tax authorities will have a licence to persuade anyone in charge of a computer bank to give them access to its simply by asserting that its contents are relevant to their work. There are two obvious kinds of dangers. First, if people fear that personal data will finish up in other people's



hands, they will be less than frank with their physicians (or physicians will try to keep important data in their heads). Second, police and government departments, notoriously ignorant of the niceties of psychiatric illness for example, and capable of prejudice (to say the least) about people's sexual history, are likely to use confidential medical data unjustly. Nothing less than the absolute prohibition of disclosure of medical records will suffice.

The practical problems of administration also need more attention than they have been given. The first time round, the British Government's bill was found not to have allowed for the special problems of mailing houses or of employers maintaining payroll records for their employees. Nothing is said in the new version of the bill about educational establishment (but the referees for scientific journals will be glad to know that their identities will not now have to be disclosed when authors demand to see computer records under their names). The international dimensions of the legislation have not been fully worked through, while it is also plain that the bill (like the European convention that has provoked it) cannot have anticipated the technical developments that still lie ahead. This is why the new bill is only a first approximation to a satisfactory piece of legislation. □

Europe's fast reactors

The British Government has thrown its fast reactor future in with Europe — twenty years late.

AFTER two years of indecision, the British Government last week decided to negotiate with its obvious European partners (Belgium, France, Italy, the Netherlands and West Germany) about the future development of fast reactors. So much was announced last week by Mr Peter Walker, the British Secretary of State for Energy. More should be known of what the government has in mind, and of how it has been driven by events to this decision, when the annual report of the UK Atomic Energy Authority is published (on 15 September). The essence of what has happened is, however, already all too plain. Like the dog in the fable which grabbed at the bone beneath the water he was crossing only to find that it was the reflection of the object he was already carrying, the British nuclear establishment has been too greedy.

A quarter of a century ago, when the European Atomic Energy Community (Euratom) was alive and formally separate from the European Economic Community, cooperation on the development of fast reactors was widely canvassed. In the event, experience showed that Euratom would be an unsuitable sponsor for such a gigantic undertaking, and the attempt to organize some kind of collaborative effort fell to the European Nuclear Energy Agency, an offshoot of OECD (then the Organization for European Cooperation and Development). In the early 1960s, the agency made the case that development would be more expensive than any single member state could reasonably afford. Smaller countries were inclined to listen, but Britain, France, Italy and West Germany preferred to stand alone.

With the passage of time, many of the potential collaborators have taken a minority stake in the French development of the Phénix reactors, but with the understanding that separate development (such as in West Germany) is not prejudiced. Britain has, however, gone its own way, building a technically superb demonstration fast reactor in Scotland, working out the technology of the enriched uranium/plutonium fuel cycle — and then discovering (a year ago) that the economic case for pushing ahead alone could not be sustained.

This is exactly the outcome foreseen more than two decades ago. This is galling even for those who then set their faces against collaboration. Their calculation then was that they were so far "ahead" that collaboration was unnecessary. The moral, now, should be that nothing can be lost by sensible international collaboration at the early stages of huge technological projects. In Europe, at least, the lesson seems to have been learned in the development of thermonuclear fusion technology. Now, governments may be more willing to follow suit in other fields in which they foot most of the bill for technological development. □

Self-defeat for universities

The threat of further budget cuts will frighten British universities. Will it shake them up?

MANY British universities have been supposing that their troubles would be at an end during the academic year beginning a year from now, when the progressive reduction of their budgets decreed two years ago would have worked its way through the system. Last weekend, on the eve of the Prime Minister's seminar (see page 172), they were rudely disillusioned. Sir Keith Joseph, the mercurial Secretary of State for Education and Science, let it be known that the general assumption that university budgets would be stabilized at the level now fixed for 1984–85 could be overtaken by events. In reply to a request from the University Grants Committee for some assurance about future budgets, he acknowledged "some hopes" that budgets would be stabilized from September 1985, but then went on to suggest that universities should also consider how best to plan their futures if their total income from public sources were reduced by a further 5–10 per cent by the end of the decade and perhaps by a further 5 per cent during the 1990s. The Association of University Teachers has quickly cried "foul".

Closer examination will unfortunately show that the universities, and in particular the University Grants Committee, are at least as much to blame. It is now more than a year since the minister wrote to the committee asking, in effect, for its views on the future of the British university system. Breaking with precedent, the minister chose to publish his letter, putting the committee on public notice that he expected a constructive answer. His letter a year ago acknowledged that consultation with the universities would be necessary, and that the development of a long-term strategy would be a time-consuming process. The committee's reply in July this year was, however, couched in such cautious language as to invite an impatient response. The committee's chairman, Sir Edward Parkes, promises a round of consultations with the universities, the submission of advice in parallel with that of other bodies "with an interest in higher education" and a final determination of a strategy by the government. To assist the first part of this process, Sir Edward asks for guidance on the budgets that will be available. He says the committee will need at least a year to decide what it wants to say.

The obvious weakness in this approach is its passivity. The committee, constitutionally the interface between the university system and the government has led with its chin. Although it is itself responsible for having devised the pattern in which different kinds of British universities are being compelled to contract, it fails to use even this occasion to say what its objectives were two years ago, and whether it considers them to be valid still. Nor does it let slip an inkling of its views on the several important issues that have arisen in the past two years — the proper balance between research and teaching in higher education, between the benefits of excellence and of diversity and between educational and vocational objectives and the propriety of increased dependence on external sources of funds. The result is what any reasonably guileful person would expect — Sir Keith Joseph has made the points himself. Let there be more diversity, of institutions and of courses, let money be channelled into the useful activities wherever possible.

The implications of all this are not, however, as serious as they may seem. There is nothing to suggest that Sir Keith Joseph would wish to take on the administration of British universities, or even that he would be able to do the job assisted only by his officials. His stern letter is therefore best read as a roundabout set of guidelines for Sir Peter Swinnerton-Dyer, who succeeds Sir Edward Parkes on 1 October. Even the threat that future budgets are insecure is not as real as it seems. The letter merely says that the government may be looking for a reduced cost of teaching individual students, not quite the same thing as a reduced budget overall. Properly regarded, this is an opportunity as well as a warning. The University Grants Committee should do what it can to make the best of it — and should not wait a year. □

US science education

Will Reagan foot the \$830 million bill?

Washington

THE steep decline in the quality of pre-college education in mathematics and science in the United States requires that the federal government should reach deep into its pocket. That is the unwelcome message for the Reagan Administration contained in the final report* this week of a commission set up by the National Science Board — the policy-making body of the National Science Foundation (NSF).

After 17 months of work, the commission has put together an ambitious 12-year plan designed to make American science education the best in the world by 1995. It would entail retraining more than a million teachers, modernizing the school curriculum and changing school timetables so that children receive more mathematics and science from an earlier age. At the heart of the scheme would be the creation of 2,000 exemplary mathematics and science schools as "landmarks of excellence".

The commission says that none of this will be achieved without the creation of new planning machinery at the federal level and a massive increase in federal spending on education. Although states and local districts would continue to be responsible for the bulk of education spending, the commission wants President Reagan to set up a national education council to oversee the spending of an extra \$955 million in each of the first three years of the scheme, \$680 million for the next two years and \$331 million for each year thereafter.

Under the scheme, school timetables would be changed to ensure that every child, from the beginning of elementary school, is taught at least an hour of mathematics and half an hour of science a day. By 1985, no student would be allowed to graduate from high school without having completed three years of high school mathematics, including a year of algebra, and at least three years of science and technology including a term of computer science.

Students going on to higher education would eventually be expected to have completed four years of high school science including physics, chemistry and a year of computer science, and four years of mathematics including a second year of algebra and courses on probability and statistics.

The commission is sharply critical of many existing school mathematics and science courses, which, it says, teach children to be technicians rather than problem-solvers. It wants NSF to set up a curriculum council to devise new courses and encourage school districts to drop components of the secondary mathematics

curriculum which have little importance with the widespread availability of calculators and computers.

Like previous reports on American schools, the commission describes the shortage of good teachers as education's most pressing problem, one that can only be solved by raising teachers' pay and status. A dwindling number of college students are entering teaching and a growing number of experienced mathematics and science teachers are leaving the profession. The commission proposes retraining more than a million mathematics and science teachers, at an average cost of \$3,000 each. The programme would be spread over five years, with the federal government meeting half the annual cost of \$698 million.

The report, with its call for vastly increased federal spending, is unlikely to be given a sympathetic reception by the Reagan Administration, which maintains that it has already been generous towards mathematics and science education. In its 1984 budget request the administration has restored the cuts it inflicted on the NSF education budget in 1982 and proposed an increase of \$9 million over the \$30 million appropriation which Congress insisted on in 1983. But it is not expected to support legislation before Congress which would authorize an extra \$400 million for mathematics and science education across all government agencies.

French science

Papon in print

PIERRE Papon, the director-general of the French Centre National de la Recherche Scientifique (CNRS), has done an unusual thing: he has written a book outlining the future of science and technology in France*. Not only may the reader boggle at how he possibly found the time to write a book — before his present post, he was full-time science policy adviser to the mid-night oil-burning science and technology minister, Jean-Pierre Chevènement — but its publication is a bold move for someone still closely involved in policy decisions.

However, the book is consonant with Papon's belief in open government. Also, Papon has hedged his bets. He sketches three scenarios, distinguished by the degree of "institutional viscosity" (his term) which might be facing present policy. So if the policy fails, it can be put down to high viscosity.

Nevertheless, the book is a generous and fascinating effort at outlining current Paponesque (French) thinking, and at

What all high school graduates should know according to the National Science Board report:

Mathematics

Algebra: Ability to solve quadratic equations; familiarity with permutations, combinations and simple counting problems.

Geometry: Ability to use the Pythagorean theorem and special right-angle relationships, understanding analytic geometry and vector algebra in three dimensions.

Other mathematics: Basic combinatorics; graph theory and discrete probability. Computer science, including programming and introduction to algorithms and iteration; the philosophical basis of calculus.

Physics

Understanding of the conservation of mass and momentum, of energy, the kinetic theory of gases and wave phenomena. Understanding the concepts of motion from particles to celestial bodies; understanding light and electromagnetism. Appreciation of atomic and nuclear physics, and of relativity.

Computer competence

How computers work and their uses; familiarity with one high-level computer language; use of a computer language to solve problems; the social problems and issues raised by computer technology.

In the past, the administration has shown particular hostility to suggestions that the federal government should take over from states and districts the main business of running and funding schools. It is hardly likely to welcome the commission's proposal that the lion's share of the cost of setting up 2,000 exemplary schools — some \$830 million — should be met from federal funds.

Peter David

**Educating Americans for the 21st Century.*

analysing the strengths and failures of French institutions. It also defines Papon's belief in a "neocolbertist" France. This term derives from the Colbert who founded the French Academy of Sciences in 1666. Colbert believed — like Francis Bacon — in a positive role for science in the state and society. If science and the state are not intimately linked *à la* Colbert, France is due to be eclipsed in the "economic war" that will befall the West after 1985, Papon believes.

On a longer time scale, Papon sees Japan losing steam by the year 2000, for lack of basic science, to be challenged in the East by China; in the West he sees the United States reasserting itself. Present French science policies would make France the third strongest technological power by that date, he says, but they require continuous and strong political will lasting for two decades. Whether that will be forthcoming is another question, for on that time scale it will certainly have to be a will that crosses party lines.

Robert Walgate

**Pour une Prospective de la Science* by Pierre Papon (Seghers, Paris, 1983); to be fully reviewed in a forthcoming issue of *Nature*.

UK science and industry

Cans and can'ts for government

MORE than 200 representatives of British industry, the universities, the City and government gathered in London last Monday for a remarkable one-day seminar on science, technology and industry. The seminar was instigated by the Prime Minister, Mrs Margaret Thatcher, who, anxious to demonstrate her personal concern for the problems besetting British industry, was chairman for the day.

As Mrs Thatcher herself remarked, it must be doubted whether such a distinguished audience has ever been gathered in one place to discuss anything at all, let alone science and industry. It must also be doubted whether many of the participants learnt very much that they did not already know. But the seminar did at least succeed in demonstrating that the problems which have been discussed so often are now being taken seriously.

In her opening remarks, Mrs Thatcher said that government expenditure on research and development (£12,700 million over the past four years) had increased by 8 per cent in real terms compared with the previous four years. And, she said, two-thirds of the defence research and development budget is placed with industry.

Mrs Thatcher also took the opportunity to announce a fact well leaked over the past few months: the British Technology Group will lose its monopoly rights on the development of government supported research. But she insisted that, while the government can create the conditions in which enterprise and innovation can flourish, it cannot substitute for the inspiration of the inventor.

A comparison with Britain's major industrial competitors shows that private industry's spending on research and development is proportionately much less in Britain than elsewhere. The situation can be helped by reducing the burden of taxation so that industry can be profitable, a course the government is pursuing.

Speaking from the point of view of a large company, Lord Weinstock, managing director of General Electric Plc, found wide support for his view that in order to bring an invention from the laboratory bench to the market place, a champion (preferably not the original inventor) is needed to steer it speedily through the successive phases of development.

Lord Weinstock challenged the idea that Britain is uniformly bad at technology transfer: many large companies do expect and get a stream of ideas from their research staff that are the basis for innovation. Many smaller companies work on ideas originating in research laboratories of large companies. But Lord Weinstock warned against encouraging "inward investment" in Britain by foreign competitors on the grounds that the base technology often does not accompany this

sort of investment, which can also exacerbate skill shortages. This, he said, may be happening in "software factories".

Mr J. Harvey Jones, chairman of ICI Plc, one of Britain's most successful large companies, dismissed the suggestion that sources of capital are reluctant to invest in high-risk innovative projects. He stressed that innovation must be accurately attuned to the needs of the market. Several other speakers also took up the theme that inaccurate reading of markets has been the largest single cause of failure in Britain.

Mr Michael Heseltine, Secretary of State for Defence, gave a number of examples of successful "spin-off" from defence technology to the civil sector: these include



Mr Michael Heseltine and Mrs Margaret Thatcher

charcoal cloth, developed at the Chemical Defence Establishment and now used in respirators, and the original concept of the integrated circuit, which came from the Royal Signals and Radar Establishment. But it has too often been foreign companies that have reaped the benefits.

In an effort to increase uptake of defence technologies in the civil sector, the Ministry of Defence will be inviting groups of entrepreneurs with access to venture capital to seek out marketable inventions within its research establishments. The groups include Cogent, Prutec, Lazards and Barclays Bank. Mr Heseltine said that these groups will act as technology brokers between Ministry of Defence (MOD) establishments and high-tech industries. The department also intends to foster more exchanges of people between government and the private sector. Most MOD contractors are large companies which own the intellectual property rights of MOD development. Mr Heseltine said his department would arrange for young small companies, usually most likely to take on high-risk projects, in future to have access to them.

Many of the contributors to the symposium were critical of the education that British universities are offering, but Sir Rex Richards, warden of Merton College, Oxford, insisted that industry can be equally well served by a graduate in physics or a graduate in Icelandic philology. Education is to stretch the intellect, and marketing and design functions should be left firmly in the province of industry. But it should

nevertheless be made easier for university academics to venture into industry.

Sir Clive Sinclair, chairman of Sinclair Research Ltd, one of Britain's most successful consumer electronics companies, presented a thoughtful analysis of the reasons for the relative decline of much of Britain's consumer industry. In the 1950s, there was a huge and growing market for consumer products such as refrigerators and washing machines, but the British public is now sated with such products. Resident expertise in new technologies must be quickly developed.

Sir Clive was one of several speakers to advocate that all higher education programmes should include courses in technology and general business studies. He also criticized existing tax regulations which discriminate against unearned income and argued that existing arrangements for pension funds act as a disincentive to mobility.

Dr Herman Hauser, managing director of Acorn Computers Ltd, made the point that British tax rules made it impracticable to offer employees share options, which they might expect to receive in other countries. This, he said, had been a major problem for his company in attracting overseas design expertise.

Mr Kenneth Baker, "Minister for Information Technology", expanded on the general theme that the role of government is solely to provide the right climate for innovation, and cited as an example the ending by the government of the monopoly of British Telecom. He boasted that cash expenditure on science and technology in the Department of Trade and Industry has steadily increased.

On private support, there was general optimism that, whatever the situation in the past, there are now a number of competent venture capital companies in Britain expert in financing high-risk projects with long lead times. Lord Caldecott, chairman of Investors in Industry, said that staffing costs in such companies are necessarily very high, but are justified by their successes.

Many speakers expressed concern about public provision for university-based research. Professor John Kingman, chairman of the Science and Engineering Research Council, put it most starkly when he said that because of cutbacks in support for fundamental research, Britain is now losing valuable opportunities. Sir Keith Joseph, Secretary of State for Education and Science, had earlier said that he would endeavour to ensure that the quality of provision was not eroded, but that it was necessary to be selective in deciding what areas were most deserving of support.

Many of the themes followed at the seminar cover well-worn ground, and some participants had reservations about whether it had been a useful exercise. But, as one research council officer remarked afterwards, "it will have been worthwhile if ministers get the message about the state of the science base".

Tim Beardsley

Nuclear weapons

US research put to vote

Washington

A STATUTE that would ban nuclear weapons research in the city of Cambridge, Massachusetts, has been put forward by anti-nuclear groups and will be decided at a referendum in November. If the proposed act is passed and then made to stick, the city of Cambridge would be compelled to fine or imprison individuals defying the prohibition. As well as Harvard University and the Massachusetts Institute of Technology (MIT), the city of Cambridge includes within its boundaries one of the leading nuclear weapons laboratories in the United States, the Charles Stark Draper Laboratory, now strictly independent of MIT, which previously gave it shelter.

The unprecedented statute, drafted by a local anti-nuclear group called Mobilization for Survival, raises far-reaching constitutional issues and is being regarded as a grave threat by the Draper Laboratory, which devotes the bulk of its research to the development of guidance and control systems for American ballistic missiles. Lawyers for both sides were expected to appear before the Massachusetts supreme court this week to decide whether the act, which has already been supported by the required number of signatures, is to be allowed to appear on the November ballot.

Under city law, referendum questions supported by a petition signed by more than 4,500 voters can be added to the ballot papers in the biennial election for city councillors. Mobilization for Survival, which has gathered more signatures than it

year, would be classified as illegal.

Several towns without any nuclear facilities have this year declared themselves "nuclear-free zones", and a non-binding call to prohibit nuclear weapons research was supported by 74 per cent of Cambridge voters in a referendum last year. But the new measure, the Nuclear-Free Cambridge Act, is the first attempt by the anti-nuclear movement to force a city council to outlaw existing facilities.

Under the act no person, university, corporation, laboratory or other institution would be allowed after October 1985 to engage in any work related to the research, development, testing, transportation or disposal of nuclear weapons or their components. A violation of the act would be punishable by up to 60 days in prison or a fine of up to \$5,000. Basic research whose "primary purpose" is not to produce nuclear weapons is explicitly exempted, as

is nuclear medicine and the use of fissionable materials for smoke detectors and other peaceful purposes.

Neither Harvard nor MIT has yet taken a formal position on the referendum. Spokesmen for both institutions said they had been advised that the act would not affect them since they already prohibit classified research. Individuals at both institutions have nevertheless joined in the controversy on both sides. Ernest May, professor of government at Harvard, is chairman of a group called Citizens Against Research Bans which opposes the statute.

Unlike earlier versions of similar legislation proposed elsewhere in the United States, the Cambridge act contains a section giving its justification for the measure. Weapons research, it says, makes Cambridge a prime target in a nuclear war and brings with it an "undesired security apparatus" that threatens civil liberties. In adopting the ordinance, it adds, Cambridge citizens would end their "complicity" with preparations for nuclear war. **Peter David**

Three Mile Island

Damage to core revealed

Washington

SONAR mapping of the interior at Three Mile Island last week has shown that the 1979 accident caused more extensive damage than earlier believed to the fuel core of the reactor. Virtually the whole surface of the core appears to have collapsed into a bed of rubble, leaving a void five feet deep and dimming remaining hopes that some of the fuel assemblies had survived intact and could be removed in one piece.

Engineers have known since making a "quick look" television inspection of the core last year that upper portions had collapsed, but did not believe that the damage extended across the whole area. The results of sonar mapping now suggest that the void extends virtually to the edge of the core and that there are unlikely to be any assemblies intact.

The discovery will put a further wrinkle in the \$1,000 million recovery effort, but may not provide any new insights into the accident itself. Site engineers say that the evidence of extensive damage could indicate that temperatures in the core had reached a higher level than hitherto estimated, but insist that there is still no sign that any fuel melted.

Further light may, however, be shed on the accident after analysis of the first sample of debris from the core, removed last week for shipping to the Department of Energy's National Engineering Laboratory in Idaho. The one-cubic-inch sample was brought to the surface by technicians using a steel scoop attached to a 45-foot rod. Unshielded, the sample would have exposed the technicians to a dose of 3 rem an hour. A total of six samples are to be removed from the core to help planners decide how

to design the tools needed to defuel the reactor.

The General Public Utilities Corporation (GPU), the reactor's owner, hopes to begin defuelling the core in March 1985 and to restore the plant to a "normal radiological condition" by 1988. Nearly a million gallons of radioactive water have already been removed from the plant and recent activities have concentrated on reducing radiation in the containment building — now down from 350 to 200 millirems per hour on the ground floor.

GPU hopes that the reactor head can be removed early next year and the plenum lifted off several months later. Rubble in the fuel core will be removed underwater into canisters in 1985 and 1986.

The Nuclear Regulatory Commission (NRC) is meanwhile showing no signs of acceding in the near future to GPU requests for permission to restart the undamaged Unit 1 reactor at Three Mile Island. Technical problems associated with Unit 1's steam generators are still unresolved and the commission is still considering charges that the utility's management lacks the integrity and competence needed to operate the reactor safely.

One commissioner, Victor Gilinsky, has already made it clear that he is not prepared to approve a Unit 1 restart until the top management of GPU has been replaced. In a memorandum last February, Gilinsky described GPU as a company whose management took a "narrow and grudging" view of its public responsibilities and sought to get by with the minimum, "be it in terms of plant equipment, staff discipline and training, or forthrightness with public authorities". **Peter David**



needs, is asking the supreme court to force the council to put the act on the ballot: at an earlier meeting the council had been unable to decide what to do about the petition.

Joseph O'Connor, vice president for administration at Draper, said the laboratory believed the act would be challenged on constitutional grounds because it was an encroachment on intellectual freedom and attempted to force a city government to thwart nationally determined policies. If it were passed, the bulk of the work conducted at the laboratory, which employs 1,800 staff and spends about \$140 million a

Australian science budget

Election promises not kept

Canberra

THE first budget of the Labor Government under Prime Minister Bob Hawke, presented on 23 August, was parsimonious in expenditure on science and technology, with most appropriations failing to match inflation. Principal beneficiaries are companies willing to invest in research in high technology. The overall economic strategy seems to be a mildly stimulatory budget constrained by containing the deficit below \$A8,400 million (4.7 per cent of gross domestic product).

Estimated government expenditure in areas relevant to research is shown in the table. As the inflation rate is about 10 per cent, any change in expenditure less than this must be regarded as a fall in real terms. The main points of the budget are:

● **Defence** science and technology establishment funds have fallen. These go to projects such as the Jindalee over-the-horizon radar, defence against anti-ship missiles (Project Winnin) and underwater detection aids.

● **Medical** research grants have risen significantly in real terms. These provide funds for basic research in universities and institutions such as state government departments, the Howard Florey Institute and the Walter and Eliza Hall Institute.

● **Industrial** research and development incentives schemes have been improved to support high technology research and development. Under the scheme, companies can apply for three kinds of grants: commencement grants, intended to encourage companies developing a new capability; project grants, for specific projects; and public interest grants for projects considered to be in the national or public interest. The biggest increase is in the allocation to public interest grants, rising from \$A4.9 million to \$A10 million.

● The Australian Atomic Energy Commission's allocation includes \$A1.6 million for the fourth year of a five-year safety upgrading programme for the Hifar reactor and \$A1.3 million towards the

building of a pilot fabrication plant to produce and test the Synroc radioactive waste disposal technique.

● The Commonwealth Scientific and Industrial Research Organization (CSIRO) has again suffered a cut in funding, particularly in its expenditure on major capital works as work on the Australian National Animal Health Laboratory nears completion. The \$A316.7 million breaks down to \$A268.7 for operations, \$A4.5 million for equipment, \$A5.4 million for repairs and maintenance and \$A26.0 million on capital works. In addition, CSIRO will receive as separate allocations \$A3.5 million for the Australian telescope (commenced last year and expected to cost \$A29.0 over five years), \$A7.0 million for an oceanographic research vessel being constructed as a national facility, increased support for key technology areas and an allocation for the construction of marine science laboratories, which will bring its total allocation to \$A331.6 million.

● The Antarctic Division's budget is increased, but by less than the increase promised during the election earlier this year. The increased support will buy transport facilities including a marine research ice-breaker and Hercules aircraft.

● **Basic research.** There is only a marginal increase, if any, in the Australian Research Grants Scheme despite an election promise of a 10 per cent increase in real terms. Together with the Queen Elizabeth II Fellowships, the scheme will administer \$A21.8 million in 1983-84 for basic research projects in physics, chemistry, biology, earth sciences, engineering, applied sciences and humanities. Marine science research administered by the Marine Research Allocations Advisory Committee, on the other hand, will get an increase. Support available to universities through the National Biotechnology Scheme amounts to \$A1.5 million, but the National Research Fellowship Scheme has at least got off the ground. **Vimala Sarma**

West German budget

Science beats inflation

WEST German non-nuclear energy specialists will have to tighten their belts a notch or two next year, to judge by budget proposals now being debated in the federal parliament in Bonn.

The ministry for research and technology (BMFT), which accounts for the lion's share of federal research cash and spends 40 per cent of its funds on energy research and development, has been squeezed on three sides. First it faces rising costs in its fast-breeder and high-temperature reactor research programme: second, it has to respond to "green" pressure to act on acid rain; and last it struggles within tight restraints on the whole federal budget. Responding to the latter, BMFT is meekly proposing a one per cent increase (in current marks) in its total budget — which means a real reduction of 2-3 per cent with present German inflation. Overall, given parliamentary approval, the renewable energy and conservation research budget of BMFT, often held up as an example by proponents of alternative energy in other countries, faces cuts of 10-15 per cent next year. Many items, such as passive solar collectors, will receive reduced support, although some, solar voltaics for example, will enjoy increases.

BMFT officials were quick last week to point out the rationale behind these moves. Many alternative energy technologies need little further research, they said: their adoption is now not a matter of research but of price and market economics.

On the nuclear side, BMFT is proud of its agreement last April with industry to support about one-quarter of the cost of the SNR-300 fast breeder project at Kalkar and of the high-temperature reactor (HTR) project. SNR-300 is now estimated to cost in total DM 6,500 million (£1,600 million); the HTR, DM 4,000 million (£1,000 million). The latter should be ready for commercial operation in October 1985, and the fast breeder around a year later.

Given the level of industrial support indicated in April, this should leave BMFT picking up a bill for Kalkar in 1984 of around DM 450 million and for the HTR of about DM 300 million including research done at Karlsruhe research centre. In addition, a large part of Karlsruhe's research budget of more than DM 300 million is spent on fast breeder research. All these figures, however, are approximate and subject to parliamentary debate, BMFT emphasizes: it is not inconceivable that the industrial contribution could rise a little, officials say.

Meanwhile, the Max-Planck-Gesellschaft, which supports some of the most important German research institutes, should do a little better than inflation (up 5 per cent in current marks), and

	1981-82 Actual \$AM	1982-83 Actual \$AM	1983-84 Estimate \$AM	Change \$AM/%
Defence science & technology establishments	125.9	138.8	144.0	+ 5.2/+ 3.7
Universities	1,006.6	1,090.9	1,126.3	+ 36.0/+ 3.3
Medical research grants	25.6	29.6	38.0	+ 8.4/+ 28.3
Walter & Eliza Hall Institute	1.6	3.2	8.3	+ 5.0/+ 157.1
Industrial research and development	24.2	52.8	71.6	+ 18.8/+ 35.6
Australian Industry Development Corporation	—	45.5 (credit)	12.5	+ 58.0/NA
Australian Atomic Energy	37.8	36.4	38.3	+ 2.0/+ 5.4
CSIRO	293.5	238.2	316.9	-11.3/-3.4
Antarctic Division	21.8	32.1	36.2	+ 4.1/+ 12.7
Research grants	20.1	22.1	24.9	+ 2.3/+ 12.8
Energy research and conservation	15.8	18.0	19.4	+ 1.4/+ 7.6
National biotechnology	—	—	1.5	+ 1.5/NA
National research	—	—	0.6	+ 0.6/NA

Source: Budget Statement No.3 of 1983-84 Budget Paper No.1, tabled in the House of Representatives on 23 August 1983. NA, not applicable.

"big science" appears to have won a battle with a 15 per cent increased allocation to major projects. Two are singled out for partial funding in 1984: HERA, a large electron-proton collider for the DESY laboratory in Hamburg, and BER II, a research reactor for Berlin. Two others are put on hold: SIS, a heavy ion synchrotron for GSI Darmstadt and a spallation neutron source for Jülich. **Robert Walgate**

UK biotechnology

Celltech signs up

CELLTECH Ltd, the British biotechnology company, is further strengthening its commercial links with Japan. Celltech has granted licences to the Sankyo Company of Tokyo which give it world-wide marketing rights for two therapeutic products that will be developed at Celltech's laboratories in Britain. Celltech will receive licensing fees, development costs and royalties.

The two products are human tissue plasminogen activator and calcitonin, a hormone involved in calcium regulation. Celltech has already cloned genes for both molecules in bacteria and hopes to have the products on the market within four years. Sankyo will be responsible for scaling up production on commercial levels. The two companies will also collaborate in research on katalcalcin, a recently-discovered hormone with properties similar to calcitonin.

Tissue plasminogen activator (t-PA) is a thrombolytic agent that has potential to replace urokinase and streptokinase, both of which have the disadvantage that their action is relatively unspecific and that they may stimulate a troublesome immune reaction. The Japanese market for thrombolytics is valued at £100 million a year, although such products are much less widely used in the United States. But a cost effective replacement with the advantages of t-PA might change that. Celltech is not alone in the race: at least two other companies, Biogen in Switzerland (collaborating with Fujisawa in Japan) and Genentech Inc. in the United States are also working on human t-PA.

Calcitonin is at present used in treating Paget's disease and hypercalcaemia. It may also prove to be useful for osteoporosis, which is a disease of considerable economic importance, being the cause of many bone fractures in older women.

In July, the state-backed British Technology Group's stake in Celltech was reduced from 44 to 28 per cent, and Celltech has successfully attracted specialist investors. The latest announcement will be seen as further evidence of Celltech's determination to reap the financial rewards of its expertise. Celltech coyly let slip at the announcement of the agreement that it has turned down several offers for similar deals with "other international pharmaceutical companies".

Tim Beardsley

Artificial sweeteners

Sour welcome for aspartame

THE atmosphere was far from sweet at last week's British launch of G. D. Searle and Company's new artificial sweetener, aspartame. Professor Richard J. Wurtman of the Massachusetts Institute of Technology has raised questions about the effects on brain chemistry of high doses of aspartame, and the Searle representatives at the launch were prepared for a fight.

Aspartame (a dipeptide of aspartic acid and the methyl ester of phenylalanine) is one of three new high intensity sweeteners approved for unrestricted use in Britain on 6 September, and Searle expects to capture a large part of the UK market for low-calorie sweeteners. Aspartame is already on the market in several countries (including the United States, Canada and France) and Searle is plainly delighted with its reception. The product is claimed to taste like sugar, which should give it a substantial advantage over saccharin. Searle's patent on the use of aspartame expires in 1987. Small wonder, then, that Searle disputes the significance of Wurtman's results.

Wurtman's data (*New England Journal of Medicine* 18 August) show that high doses of aspartame given to rats produce a rise in brain levels of aromatic amino acids (including phenylalanine) and that the effect is potentiated by glucose. Aspartame blocks the rise in serotonin levels that normally follows glucose ingestion. Wurtman suggests that aspartame, while not toxic in the usual sense, may affect mechanisms controlling carbohydrate and protein intake and raise phenylalanine levels dangerously in susceptible individuals. Although other foods may contribute much larger amounts of phenylalanine (Phe) to the diet, they contain other amino acids that compete for access to the brain and so limit brain levels of Phe.

Because of impaired Phe metabolism, people suffering from phenylketonuria will certainly have to be wary of aspartame. In order to avoid brain damage caused by an increase of Phe to toxic levels, severe cases must limit their intake to around 200 mg per day. A circular produced by Sir Henry Yellowlees, Chief Medical Officer at the Department of Health, points out that soft drinks sweetened with aspartame may contain up to 400 mg of Phe per litre.

Searle has voluntarily announced that it will include a warning to phenylketonurics on its own aspartame product and will encourage other manufacturers to do likewise. As phenylketonurics all know that they have the disease and have anyway to follow an extremely restricted diet, it will be possible for them to avoid aspartame.

The real controversy is whether other groups could be affected. Wurtman is not concerned about low levels of aspartame — he uses the product himself — but says that the behavioural tests necessary to deter-

mine the effects of high doses have not been carried out. A child drinking soft drinks in large quantities will, he says, approach levels of phenylalanine equivalent to those in his experimental subjects. Wurtman says these levels are well within the range that might produce behavioural effects. Other authorities are more sceptical, saying that the changes Wurtman has observed are within the range of normal variation. But if he is right, individuals heterozygous for the phenylketonuria gene, who have a slightly impaired Phe metabolism, might be more at risk than others.

Wurtman's conclusions have been rejected by the US Food and Drug Administration, but Wurtman maintains that his points have not been answered. The data have not yet been formally considered by the UK Department of Health's Committee on Toxicity, but Labour Member of Parliament Jack Ashley has asked for an emergency meeting to discuss aspartame. The committee will otherwise next meet in October. Searle says it is fully confident that the authorities will have no reason to reconsider their decision. It is also pointed out that no ill-effects have been encountered with the product in the many countries where it is in use. **Tim Beardsley**

Mediterranean treaty

SOME 85 per cent of all sources of pollution in the Mediterranean are now under control, at least on paper. Last month the 1980 Athens treaty on land-based sources of pollution, inspired by the United Nations Environment Programme (UNEP), came into force with its ratification by six of the original sixteen signatories.

The six ratifiers are Algeria, Tunisia, Egypt, Turkey, Monaco and France, which, through the Rhône river, is one of the major industrial and agricultural polluters of the Mediterranean. The remaining ten signatories are near ratification, says UNEP. Full implementation of the treaty would cost some £7,000–10,000 million, UNEP estimates, and it expects that money to be spent in the next 10–15 years.

According to Dr Mustafa Tolba, executive director of UNEP, pollution monitoring shows that because of untreated sewage, 20–25 per cent of Mediterranean bathing beaches are now unsafe for bathing, and that only 4 per cent of shellfish-growing areas produce shellfish "safe for direct consumption". Some coastal waters are also showing signs of eutrophication, Tolba says. The resulting loss of revenues from tourism and fishing are difficult to estimate, but it seems that the threat of such losses is now the chief incentive to ratify the Athens treaty.

Robert Walgate

Prejudiced reporting?

SIR — Because any doubt thrown on the integrity of a scientist has serious consequences, such allegations should be dealt with only with extreme caution, and the rule underlying any democratic society, that any person should be held innocent until proved guilty, should be scrupulously followed. In my opinion, the spirit of the articles published in *Nature* on the investigation involving the research of Dr Karl Illmensee does not fully conform to that rule.

Like many others, I was shocked to hear that doubts had been cast on the credibility of a well-known scientist. The information published in *Nature* (2 June, p.363) and *Science* (3 June, p.1023) on the subject was that suspicions were being harboured against Dr K. Illmensee and that committees had been appointed in Geneva and Bar Harbor to investigate the case. However, although it was not explicitly stated, the extensive coverage of the case in *Nature* in a news item and the reference to it in an editorial entitled "Is science really a pack of lies?" left the impression that the accusations against Illmensee were founded. This constitutes, unwittingly, the passing of a judgement against the scientist and the manipulation of public opinion.

Moreover, I was surprised to witness the relative ease with which many scientists accepted the truth of the charges (or even rumours before these publications) against a colleague, without knowing the relevant facts. This, in my opinion, is a disturbing symptom and may reflect low standards of the mutual trust which is so important for the progress of scientific research.

A correct attitude towards scientific honesty should make it all the more difficult to accept that a well-known scientist has resorted to fraud. The instinctive reaction to such a charge (as long as proof had not been found) should have been disbelief. In all fairness, we should give our colleagues the elementary right to a fair and respectable investigation, free of unsubstantiated and biased public opinion.

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DR YAFFE is right to insist on the principle that a person must be supposed innocent unless proved guilty but wrong to suggest that *Nature's* reporting tends otherwise. Both the articles to which he refers stem from an announcement by the University of Geneva that an internal investigation had been completed, and that steps were being taken (at the end of May) to appoint an external commission of inquiry. After more than two months, the membership of that committee was announced; it met for the first time at the end of August. Meanwhile, *Nature* has been glad to report that an investigation at the Jackson

Laboratory in Bar Harbor of Dr Illmensee's work there has exonerated him and his US colleagues from blame (see *Nature* 16 June, p.563).

For a university to report that an investigation has been found necessary is, fortunately, rare, and necessarily a matter of public interest. To have failed to report such a development and to describe the background to it would more properly have been held to be "the manipulation of public opinion". But *Nature* knows nothing substantial that it has not published, has not prejudged the issue, has not sought advance information from the members of the investigating commission, agrees with Dr Yaffe that the scientific community should also await the outcome of that investigation and hopes, in everybody's interests that it will not be much longer delayed. Justice should be swift as well as impartial.

Editor, *Nature*

Sizewell safety

SIR — Your leading article exhorting the government to "end the Sizewell agony" (*Nature* 4 August, p.382) suggested that the terms of the inquiry should be restricted to local planning issues, while the question of whether reactors of particular types are in some acceptable sense "safe", is a matter for central government. Yet is it not now widely accepted that a community should have a very major say in what risks it is to bear? The protracted inquiry is providing an essential forum for that say.

Certainly the inquiry is costly, but a wrong decision could be more so. An inquiry restricted to local planning issues would have neither the breadth nor the depth to examine the fundamental criteria upon which thorough decision making should be based.

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Steps against arms

SIR — M.C. Goodall (*Nature* 4 August, p.390) is of course free to make his own deductions about what the scientific establishment is so dismally doing to influence the arms race. The impression given by his letter is that he is dismally unaware of what is happening in this field. The Pugwash movement has just held a conference on "Avoiding Nuclear and Other Wars and Reversing the Arms Race".

The Pontifical Academy organized recently a number of meetings that led to a Declaration of the Prevention of Nuclear War adopted in the Vatican by representatives of 36 National Academies of Science and of international scientific organizations.

The special number of *Ambio* (Vol. XI Nos 2-3, 1982), 'Nuclear War: The Aftermath' was also the work of scientists under the sponsorship of the Royal Swedish Academy of Sciences. There have been other activities by national groups of scientists, with another at the end of October in Washington DC on "The World After Nuclear War".

The International Council of Scientific Unions (ICSU) at its 19th General Assembly in 1982 resolved to make an assessment of the biological, medical and physical effects of the large-scale use of nuclear weapons — this is just beginning — and ICSU's Scientific Committee on Problems of the Environment is already undertaking a study of the environmental consequences of nuclear war, the first stage of which will concentrate on the atmospheric aspects.

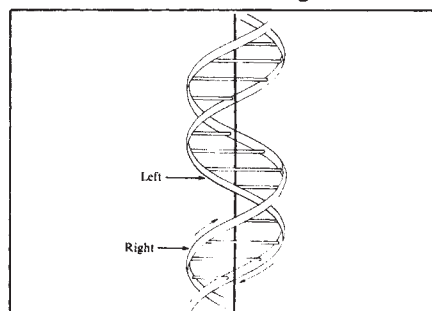
Perhaps the scientists are dilatory and dismally doing little — but do other professional groups have better track records?

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Novel DNA structure

SIR — We look forward to *Nature's* Symposium: Molecular Biology Now & Tomorrow. If the advertisement that has appeared in many issues of this journal is any indication, the conference should provide real excitement. We find it especially amazing that after 30 years DNA continues to yield basic structural secrets; we refer specifically to the structure of DNA depicted in the advertisement for this symposium. Who would have thought that one strand could become left-handed while the other strand remained right-handed?



Since this structure appears as yet unnamed, may we suggest it be called E-DNA in honour of the great illusionary artist M.C. Escher. Leave it to *Nature* to usher in Escher DNA?

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Good cause for celebration

Next week's conference to celebrate the thirtieth anniversary of the discovery of the structure of DNA in Boston is more than an exercise in hagiography.

WHAT more can there be to say by way of celebration of the publication of the structure of DNA (J. D. Watson and F. H. C. Crick *Nature* 171, 737; 1953)? That is a proper question. *Nature* held a conference on the theme in Cambridge (England) earlier this year. Why now another (next week) in Boston, Massachusetts? Is there not a danger that the topic will be done to death? Or that endless rounds of celebrations will endow particular events with a spurious importance, distracting people's attention from the simple truth that even the most striking discoveries are as much the products as the determinants of the intellectual climate of their times?

Of course. And nobody has an interest in making more of a thirty-year-old discovery than the circumstances warrant. But the circumstances are remarkable. Rarely can there have been an occasion when the effect of a discovery has been to provide so quickly a persuasive answer to a question in the forefront of many people's minds: what is the genetic material, and how does it work?

Newton's theory of gravitation was a welcome surprise to some of his contemporaries, but a matter of indifference to most of them. Darwin's evolution, widely recognized as important, had to be rammed down the throats of many of his contemporaries. The significance of Planck's quantum went unrecognized (even by Planck). Einstein's special relativity, even with its echoes of earlier work by Lorentz and Poincaré, made sense to some people but nonsense to others. Even the development of the quantum theory in 1925 and 1926, which predicated the most radical revision of the conception of physical reality (not yet complete) but which also made possible what now passes for physical science (not to mention the electronics industry), was blunted, at the time, by confusion — Schrödinger's sneaking hope that wave mechanics would reconcile the inconsistencies of classical mechanics, the lack of a language (whose grammar Dirac provided), conceptual disputes (which persist) and sheer complexity.

The effect of the publication of the correct structure for DNA was much more immediate. If major discoveries are keys to locked doors, this key has been quickly fitted to its lock and the door has swung open on well-lubricated hinges. Much of the pleasureable excitement of the past thirty years has been the way in which simple expectations of nature have been repeatedly confirmed. If DNA is the genetic material,

there must be a biochemical mechanism by which it replicates faithfully (J. D. Watson and F. H. C. Crick *Nature* 171, 964; 1953), and, indeed, there is (Kornberg, 1956, M. Meselson and F. W. Stahl *Proc. natn. Acad. Sci. U.S.A.* 44, 671; 1958). If genetic information rests in the arrangement of consecutive nucleotides along the length of DNA molecules, there must be a code which, at its simplest, will be a triple code; right again (F. H. C. Crick *et al. Nature* 192, 1227; 1961). And there must be some kind of messenger for carrying genetic information from the nucleus to the cytoplasm of a cell (S. Brenner, F. Jacob and M. Meselson *Nature* 190, 576; 1961) . . . and so on. Along the way, inevitably, there have been surprises, reverse transcriptase for example. And nothing of what has been accomplished in the past three decades would have been possible without the literally marvellous techniques that have been developed and which have led to the deliberate manipulation of genes and their elements. (See page 189 for the first functional synthetic chromosome.) Can such a torrent of excitement be over-celebrated?

To whom, or what, should go the credit? The two obvious recipients are by now satiated with congratulation, but it is easy to forget that, thirty years ago, confusion about the genetic mechanism was not much less serious than the confusion now about the strong nuclear interactions (is there a *top* quark and all that?). How otherwise could Avery's early experiments, in retrospect a convincing demonstration that DNA can transform the genetic character of organisms, have made so slight a mark on the climate of opinion? (The Royal Society awarded Avery its Copley Medal, showing that the importance of his work was recognized, but the citation referred to the "introduction of chemical methods in immunology", showing that its meaning was not.) Part of the trouble was the hankering after the notion that proteins are the genetic material, which no doubt is why Chargaff's demonstration of the constancy of the nucleotide ratios (A to T and C to G) was at first regarded as a puzzle, not part of an explanation. Watson and Crick's success was twofold: their model was simple, but it embodied not merely a means for encoding genetic information but the means for generating the mechanism of its own replication.

What happened in the decade after 1953 is too easily forgotten. The door may have swung open, but there were still many who

suspected it was the wrong door. Biologists were reading the wrong books. Schrödinger's *What is Life?* is much quoted as an antecedent of the double helix. Von Neuman's work, now flourishing under the banner of "cellular automata" (see S. Wolfram, *Rev. mod. Phys.* 55, 601; 1983) would have been a more robust guide to the logical requirements of self-replicating systems. In the event, it was Crick, egged on by Gamov's speculations (*Nature* 173, 318; 1954) who improvised the logic characterizing the genetic code before its existence had been demonstrated. It remains a triumph of imagination over the inconsistencies and gaps in data that he and Brenner should have postulated the existence of adaptor molecules, now called transfer-RNA, before they were in due course found. The door would have been left to swing open in the wind had it not been for the proselytizing zeal in the 1950s of a small band of people devoted to the principle that even biology is not so complicated that it cannot be understood.

None of this should be mistaken for an arrogant supposition that there are no problems left. Plainly that is far from true. Even the mechanisms of DNA replication and transcription are not yet known in sufficient detail to satisfy normal and practical curiosity, let alone the purists. The need also to understand how messenger molecules are processed, which is not yet possible, has become apparent only in the past decade. The control of genes in higher organisms is far from understood. Little worth knowing is known in molecular terms about the process of cellular differentiation. Molecular biology has so far contributed to the problem of the origin of life principally the conviction that the problem will prove soluble. That a biotechnology industry is now being built is not surprising (which is not to demean the ingenuity of those who saw how that might be done), but the tasks now being attempted will almost certainly be swamped by those not yet imagined. What has changed for certain, and permanently, is the style of biological research. Asking the right questions (those which can be answered simply) is the objective. Simplifying experiments have come to complement observation. The expectation that answers to the right questions will be generally useful has been widely sustained in the past thirty years. That is the cause for celebration.

John Maddox

Oceanography

States of past oceans

from Tjeerd H. van Andel

No more than a quarter-century ago we knew little about the deposits beneath the ocean floors, and less concerning the history of the two-thirds of the world which they occupy. Since then the new discipline of palaeoceanography has come into being, and has quickly illuminated a remarkable variety of geological issues. Three factors have been chiefly responsible for the prosperity and rapid growth of the new field: the reconstruction, with the recipes of plate tectonics, of past arrangements of continents and ocean basins; the vast collection of ocean sediment cores gathered by the drill ship *Glomar Challenger*; and a finely detailed time scale based on pelagic microfossil zones calibrated with isotopic dates. The time scale made it possible to substitute ages in years for the usual relative stratigraphical units, such as the Cretaceous or the Pliocene, and finally enabled geologists to discuss past events in terms of their rates, an indispensable step towards understanding the dynamics of ancient oceans and climates. The current vogue for climatic and palaeoclimatic research coupled with a growing awareness of the long-term interactions between climate and oceans has helped broaden the perspectives.

So quickly did palaeoceanography develop that a synthesis became possible and necessary within a decade of the first use of the term. This synthesis (J. Kennett in *Marine Geology*, 593, Prentice-Hall, New Jersey; 1981) showed that during the past 150 Myr the oceans have occupied two distinct states. The earlier and longer-lasting one was warm, with a small Equator-to-pole surface-temperature gradient, temperate poles, a weak stratification and a sluggish deep circulation. In contrast, the present state of the ocean is characterized by a large temperature gradient from Equator to poles, by glaciated polar regions, a stable density stratification and a vigorous deep circulation driven by cold dense water which sinks around Antarctica and travels north at abyssal depths. The warm less stratified ocean seems to be the normal state; it persisted throughout the Mesozoic and much of the following Cenozoic, making way for the cold stratified state only gradually during the last few tens of millions of years. Quite possibly the warm ocean also prevailed during much of the preceding Palaeozoic and even earlier, but our knowledge of such old oceans is still very small.

It is on the transition from the warm to the cold state, beginning about 40–50 Myr ago, that palaeoceanographers have so far mainly focused. The transition was accompanied by, and probably mainly responsible

for, the onset of our Ice Age which continues to dominate the Earth's climate to the present day and will continue to do so for some time. In turn, the transition itself was surely due mainly to the closure of equatorial and opening of circum-Antarctic seaways that occupied continental drift.

A recent paper by Gerta Keller and John A. Barron (*Bull. geol. Soc. Am.* **94**, 590; 1983) illustrates how much we have learned from palaeoceanography about the processes that cause climatic changes and how far we have come since Kennett wrote his synthesis just a few years ago. Dealing with the period from 25 to 5 Myr ago which includes the Miocene, the time of the formation of the Antarctic icecap and of the development of the modern ocean circulation, the authors show that, at the beginning of this interval, latitudinal temperature gradients in the ocean were still small, the planktonic flora and fauna globally quite uniform, and the deep circulation not very active. Initiated by the separation of South America and Antarctica which disrupted the last of the land bridges in high southern latitude, a deep circum-Antarctic current developed early in the Miocene. It thermally isolated the south polar continent from the warmer waters in the north and brought about considerable cooling. The result was a wholesale extinction of pelagic life, and the establishment of new planktonic communities in a much more distinct zonal arrangement. A little later, around 15–16 Myr ago, another major change in current and sedimentation patterns was brought about by the rise of barriers in the Caribbean — followed eventually by the closure of the Isthmus of Panama — which blocked the passage of the deep equatorial current, raised the fertility of the Pacific and deflected northwards what is now the Gulf Stream. Approximately simultaneously, cool northern waters began to spill into the central Atlantic as the last connections between northern Europe and North America sank to abyssal depth. This modification to the Atlantic deep circulation led to a set of complex interactions which slightly raised the temperature of the surface waters around Antarctica, thus providing more atmospheric moisture. This increased snowfall and the Antarctic icecap was born. Eventually, the climatic consequences of these and analogous events brought about the cooling and increases in precipitation that resulted in the alternating glaciations and deglaciations of the last several million years in the Northern Hemisphere.

In outline, this course of events has been known for some time. What is novel in

Keller's and Barron's paper is the first global use of an advanced time scale which allows them to discriminate between events as close together as 100,000 yr. Although still a little long compared with the response rates of ocean and climate — an entire glacial-interglacial cycle may fit within such a time span — it is a huge step beyond the 1 or more Myr one had to be content with before. With this time scale the authors show that the key oceanic and climatic events were not gradual, as one would expect because they are dictated by the stately drift of continents and slow opening and closing of seaways, but abrupt and stepwise. Moreover, there are too many such steps. The initiation of the circum-Antarctic current, for example, although caused by the single event of the opening of the Drake Passage, took place in two sharp stages at 23–22.5 and 20–18 Myr ago. Similarly, brief abrupt alternations between warmer and colder intervals punctuate the period from 16 to 5 Myr ago, corresponding only in the broadest sense to tectonic events. One is thus compelled to consider, as students of the more recent glacial history have already begun to do, the role of factors other than continental drift, positive and negative feedbacks perhaps, or the consequences of exceeding thresholds in delicately poised systems. Although only dimly perceived at the moment, this striking behaviour of ocean and climate should have much to tell us in the long run.

A fascinating aspect of the Keller and Barron paper is the use they make of something which generally frustrates rather than assists geologists, the existence of breaks or hiatuses in the sediment column. When we began to contemplate the possibility of drilling and coring through the oceanic sediment blanket, it was widely believed that there, in the ultimate sink for continental debris, an exceptionally complete record would be found. It has not been so; the strata of the ocean floor are as full of gaps and disruptions as are those of the continents and their margins. However, in the ocean the processes that cause such gaps, a dearth of sediment because of reduced biological productivity in surface waters, erosion by bottom currents, or the dissolution of calcareous and siliceous materials by corrosive abyssal waters, are fairly well understood and seem to be less random than those that break the record on land or in shallow seas. Much can therefore be deduced from the distribution in time and space of pelagic hiatuses and Keller and Barron make trend-setting use of this opportunity.

Hardly ten palaeoceanographic years have passed and we have learned so much. Where are the next ten likely to take us? First, the high-resolution time scale which served Keller and Barron so well should be extended much further back in time. This is essential because the present resolution of a million years or so, delightful as it is compared with what it used to be, is not suf-

ficient to study oceans and atmosphere capable of changing much more rapidly than that.

This in hand, we might proceed to examine the ocean in its warm less stable state, a difficult undertaking because we lack the convenience of comparing it with a modern example. To understand the warm ocean is important, because it is the backdrop against which much of the history of life has taken place, it is the most probable state for the hundreds and thousands of millions of years that lie beyond the oldest ocean floor we can drill, and it has a practical side as well. Many of the world's most prolific sources of oil date from the Mesozoic and are clearly associated with a warm ocean of unstable stratification. Paradoxically, this ocean was probably not very fertile but, because of its sluggish deep circulation, preserved its organic matter exceptionally well in ubiquitous black shales. Such black shales appear with increasing frequency as we go farther back into the past, raising important and perhaps economic questions.

More fundamental issues lie beyond. To some degree palaeoceanography has been able to free itself from the vicious circle of deducing environments from fossil assemblages, then explaining evolution in environmental terms. With at least partial independence of the two lines of evidence, we may take a fresh look at the driving forces of evolution in the sea. There is, for instance, the sudden expansion in the middle Mesozoic of planktonic organisms with calcareous and siliceous shells, in great abundance and diversity. What brought about this sudden intrusion of life into the carbonate and silica cycles of seawater which they have dominated ever since? For the past 100 Myr these organisms have produced the bulk of the oceanic sediment blanket. It is not clear what pelagic sediments looked like before their advent. Was the ocean floor largely bare? If so, what was the impact of enhanced submarine weathering on the chemical composition of seawater?

The constancy of composition of seawater is a tenet of long standing in geology and palaeontology, albeit supported by little and mostly circumstantial evidence. If we can so clearly show that the present ocean is a poor representation of the states of most past oceans in terms of temperature, circulation, or oxygen content, should we not re-examine its chemical history as well? The recent discovery of an extensive system of deep-sea hot springs on the crests of mid-ocean ridges suggests that the answer is affirmative. These springs cool the new oceanic crust by circulating ocean water through cracks and fissures. In the process, some components are precipitated in the rock, others leached from it and the water upon exit is quite different from average ocean water. They are therefore a major, and so far unsuspected, factor in the geochemical balance of the ocean. At the current speed of plate movement the

equivalent of the entire ocean is cycled through the hot-spring system every 8–10 Myr. Speeding up or slowing down the rate of plate movement may thus alter quite rapidly the composition of seawater for key components such as carbon dioxide, calcium, potassium, ferrous iron (and hence oxygen) and perhaps even sodium chloride. Life may not have evolved in an ocean of constant composition after all. The record of the chemical history of seawater lies concealed in the altered

basalts of the oceanic crust, now crumpled into the rocks of zones of ancient plate collisions.

In all, the next decade ought to be interesting, full of surprises, quite interdisciplinary, and a worthy match for the 1960s when plate tectonics turned our orderly world vision upside down and inside out. □

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Astronomy

Ultra-high-energy γ rays from Cygnus X-3

from Neil Porter

THE binary X-ray source Cygnus X-3, when discovered in 1967, seemed normal, emitting feeble microwaves and with no optical counterpart. In September 1972, however, it flared up in radio wavelengths (but not in X rays) briefly to become one of the strongest radio sources in the sky; and a whole issue of *Nature*¹ was devoted to it. Since then it has appeared and disappeared as a γ -ray source at 100 MeV, shown γ -ray emission at 10^{12} eV, and last September flared again in radio, producing a relativistic jet structure² that was almost superluminal. Now, workers in Kiel³ have detected it as a powerful γ -ray source at energies above 2×10^{15} eV. Cygnus X-3 appears to be the strongest known γ -ray emitter in the Galaxy, over a very wide range of frequencies. The production of such extremely high-energy γ rays in such strength presents model-builders with considerable problems.

Shortly after the 1972 eruption Stepanian and his colleagues at the Crimean Astrophysical Observatory detected γ rays in the 10^{12} -eV region, using the atmospheric Cerenkov effect⁴. In this technique, the electron-photon cascade in the atmosphere emits a faint flash of light, which can be picked up optically by directional detectors on the ground. They found a variable signal with a 4.8-h periodicity corresponding to a similar known variation in the X-ray emission. A 4.8-h variation was also seen for γ -ray emission in the 30–100-MeV region by the SAS-2 satellite⁵. Some balloon flights observed the varying flux also, but others found no signal at all; neither did the later γ -ray satellite COS-B⁶. It is not clear whether the SAS-2 and COS-B results are in direct conflict, as Cygnus is a very complex region containing other γ -ray sources.

The Crimean group continued observing⁷ through the 1970s. Their results were confirmed qualitatively by three other groups^{8–10} in 1980 and 1981. All agree the signal has a 4.8-h periodicity, but the phase and duration of the signal within the 4.8 h are not clear. There is also a longer-term

variation which may be correlated with a 34.1-day X-ray cycle.

At energies of 10^{13} – 10^{14} eV, particles from an electron-photon cascade can be detected at mountain altitudes, and at 10^{15} – 10^{16} eV the particles reach sea level. Samorski and Stamm², using the Kiel extensive air-shower array, observed Cygnus X-3 during 1976–80 in the region above 2×10^{15} eV. They have detected a signal at the 4.4 standard deviation level, which when analysed shows a 4.8-h period with a peak approximately in phase with the Crimean results. The total number of excess events detected is small, only about 16, or 1 every 3 months from the direction of Cygnus X-3. Nevertheless, the effect is very significant, particularly when the 4.8-h periodicity is taken into account.

Thus a photon spectrum, approximating to a power law with nearly constant exponent, can be plotted over 11 decades of energy from X rays to these ultra-high-energy γ rays. The slope of the spectrum is extremely flat with an integral exponent of 1.1, implying a luminosity of nearly 10^{37} erg s⁻¹ between 2×10^{15} and 2×10^{16} eV, at the estimated distance of 11 kpc. The true

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energy release at these energies could be even higher, as some of the γ rays may be absorbed by interaction with the universal microwave flux^{11,12}.

Production of γ rays at 10^{12} eV has proved difficult to accommodate in a model. At 10^{16} eV the problems are even greater. Most authors accept that the 4.9-h period represents the orbit of a compact object around a massive companion star. In one model, both objects are immersed in a cocoon of material¹³ which scatters X rays, and to a lesser extent, γ rays.

To accelerate either electrons or protons to sufficiently high energies in a short time, a highly ordered system is required, and a fast young pulsar is therefore a candidate for the compact object. Vestrand and Eichler¹⁴ suggest that a beam of pulsar-accelerated particles, striking the extended atmosphere of the large companion tangentially just before and after eclipse,

generates γ rays in two narrow beams separated by somewhat less than half a period. This would give a pattern of the form found by the Crimean group. The primary particles could be either electrons or protons. Alternatively, the compact object could be a black hole¹⁵.

At 10^{16} eV, electron energy losses through synchrotron radiation would be very rapid, so that a nuclear origin for the γ rays, through the decay of π^0 or other mesons, may be more plausible. If this is the case, Cygnus X-3 should be a neutrino source, and may be detectable by DUMAND¹⁶. This is in the future. In the meantime, air-shower groups with directional arrays will be examining their data carefully, and Cerenkov groups will be seeking to improve their techniques^{7,17}. $\square$

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Theoretical physics

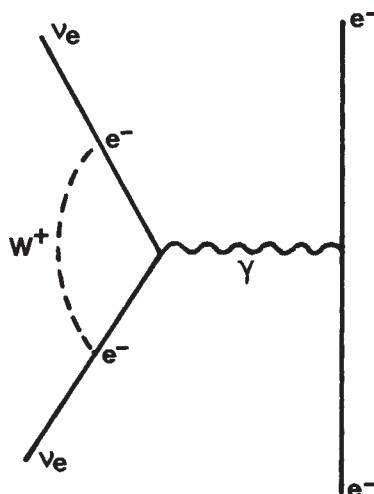
Radiative corrections in electroweak theory

from Norman Dombey

THE recent experiments at CERN which demonstrate the existence of W and Z particles^{1,2} have confirmed another basic property of the theory unifying weak and electromagnetic forces for which S. Glashow, S. Weinberg and A. Salam received the Nobel prize in 1979 (ref. 3). The theory, however, demands more than the existence of the W and Z; in order for it to be accepted with the confidence with which physicists now accept quantum electrodynamics (QED) there must be experimental confirmation of the precise relationships between the various quantities which enter into the theory. The computation of these relationships in a modern quantum field theory requires the calculation of the radiative corrections to the theory but this is substantially more complicated in electroweak theory than it is in QED. Because of the undoubted importance of verification of the electroweak theory, a small workshop was recently convened* to seek a consensus among those in the field on the way in which the theory is renormalized — that is, on how the radiative corrections are calculated.

The most important feature of the unified theory of weak and electromagnetic interactions based on the gauge group $SU(2) \times U(1)$ (the standard electroweak model) is its renormalizability. This means, as Salam⁴ has stressed since 1960, that it must in principle be possible to determine all the physical quantities encompassed by the theory — particle

lifetimes, scattering cross-sections and so on — as precisely as the basic parameters of the theory allow. This is the case in QED where, for example, the muon magnetic moment has been determined up to order $(\alpha/\pi)^3$ where $\alpha \sim 1/137$ — the fine structure constant as determined by the Josephson effect — is the perturbation expansion parameter. Measurement and theory here agree to a few parts in 10^8 which is the order of magnitude of both the theoretical and experimental error, from which fact stems the general agreement among physicists of the correctness of QED. Without inclusion of the higher-order terms of perturbation theory, which are known as the radiative corrections to the theory, there would be no such precise



One of the radiative corrections to ν_e - e^- scattering which gives rise to a term proportional to $\ln(M_W/m_e)^2$.

agreement with experiment.

The standard electroweak model is that written down by Glashow⁵ in 1961, which in its simplest renormalizable form involves just one added Higgs boson. This then gives the Weinberg⁶ mass relation between M_W and M_Z :

$$M_W = M_Z \cos \theta_W \quad (1)$$

The standard model demands not only the existence of W and Z, which the UA1 and UA2 experiments at CERN have now demonstrated, but also the precise measurement of the relationships predicted in the model between M_W , M_Z and the other parameters of the theory. The perturbation parameter in the standard model can also be taken to be the fine-structure constant since the model contains QED; hence in principle it should be possible to calculate physical quantities to the same degree of accuracy as in QED.

This, however, has not yet been done and the predictions of the theory have been verified so far only 'at tree level'; that is, only to the lowest order of perturbation theory. Thus radiative corrections (for example the figure) are not included. A theory which is verified only to this accuracy can be said to be an effective or phenomenological theory: if it were a complete field theory like QED the radiative corrections must be taken into account. In fact the radiative corrections are even more important in the electroweak model than in QED because of the large dimensionless number $(M_W/m_e)^2 \sim 10^{10}$ in the model. Diagrams such as that in the figure involve a logarithm of this number. Large logarithmic coefficients of this type do not arise in QED where the gauge particle is the photon and has zero mass. Furthermore, because of the large masses which occur in the electroweak model, there is an arbitrariness in the way renormalization is carried out: for example, should the perturbation theory expansion parameter be the fine structure constant evaluated at zero energy as in QED (or on-shell because the photon mass is zero) or should it be evaluated at the mass of the $W \sim 80$ GeV (or off-shell)? There is a related arbitrariness in the definition of the other basic parameter of the model: how should the electroweak mixing angle be defined? All definitions are equivalent at tree level but differ when radiative corrections are taken into account.

The workshop that was called to resolve these problems was opened with a paper by D. Bailin (University of Sussex) in which he outlined the history of the small discrepancy between the weak Fermi coupling constant G determined by measurement of the muon lifetime and that determined from nuclear β decays. The introduction of the Cabibbo⁷ angle in nuclear decays but not in muon decay tended to remove the discrepancy. Blin-Stoyle and Freeman⁸ in 1970, however, in a careful analysis of the nuclear data including both radiative corrections and the Cabibbo angle, showed

*Topical Conference on Radiative Corrections in $SU(2)_L \times U(1)_Y$ was held at the Centre for Theoretical Physics, Trieste, Italy on 6-8 June 1983.

that while the positive discrepancy had disappeared, a small negative discrepancy of about 1.5 per cent remained. This discrepancy could only be explained if 'very heavy intermediate bosons' of mass M existed in the theory. Then the radiative corrections to β decay not present in muon decay gave rise to a term

$$-(3\alpha/\pi)\ln(M/m_p) \quad (2)$$

where m_p is the proton mass, which removed the 1.5 per cent discrepancy for $M \sim e/G^{1/2} \sim 90$ GeV typical of electroweak theories. This result was for many years the only experimental evidence for the existence of the mass scale associated with the W and Z as Bailin and I have pointed out⁹. Bailin concluded that only when experiments can be made sensitive to effects of order $\alpha \sim 1$ per cent can the standard model be verified; this in itself demands a theory that can make definite predictions to this accuracy.

L. Baulieu (Ecole Normale Supérieure) then presented a paper which was a technical *tour de force*: he proved the renormalizability of the standard model on one transparency. He then showed the important result that for the measurable quantities (the so-called S-matrix elements) in the theory to be gauge invariant, the renormalization scheme used must define all its parameters on-shell. This property the standard model shares with QED where all S-matrix elements can be expressed in terms of the fine structure constant and the masses of the particles in the theory; it is unlike the situation in quantum chromodynamics (the present theory of strong interactions) in which all mass scales are arbitrary and renormalization is performed off-shell.

This result led directly to the appropriate definition of the electroweak mixing angle θ_w . Experimental papers take θ_w to be the mixing angle between the electromagnetic and weak neutral currents. This is a perfectly proper definition but one that leads to difficulties in a renormalization scheme since the weak current is normally defined at mass M_Z while the electromagnetic current is defined at zero mass. A. Sirlin of New York University (who first calculated¹⁰ a radiative correction to a weak process back in 1956 and who has been the leading advocate of the importance of radiative corrections since unified electroweak theories were introduced) suggested with little dissent that Baulieu's on-shell approach required the natural definition of θ_w in the standard model to be

$$\cos\theta_w = M_w / M_Z \quad (3)$$

using equation (1).

Salam was very keen that the workshop consider the question of the range of values of M_w and M_Z allowed in the standard model since he had just received a telex from CERN announcing the discovery of the Z of mass around 97 GeV. The question was answered by M. Consoli (Catania) and Sirlin who showed that even allowing a

Ornithology

Discovery of a new albatross

from P. Jouventin and J-P. Roux

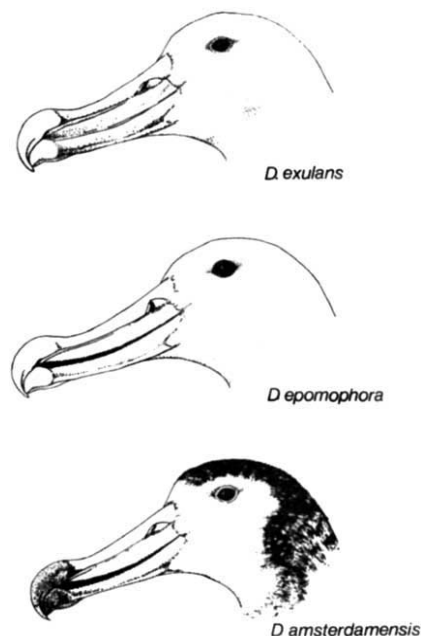
A NEW species of albatross has been discovered on Amsterdam Island, situated in the southern Indian Ocean midway between South Africa and Australia, and named Amsterdam Albatross, *Diomedea amsterdamensis*.

Several 'great albatrosses', presumed to belong to the species *Diomedea exulans* (Wandering Albatross), had occasionally been reported on the island. However, during a recent study it was realized that the population differed from the Wandering Albatross in several ways (see the figure).

The weight of the adult is nearly 3 kg less than in *D. exulans*; the breeding plumage is dark; the eyelid white instead of blue as in *D. exulans*; there is a decorative black stripe on the beak, as in the Royal Albatross (*D. epomophora*), and a black patch at the tip; and finally, the breeding cycle of *D. amsterdamensis* starts 2 months later than in populations of *D. exulans*.

The family Diomedea has been thought to comprise 13 species. Ernst Mayr (personal communication) believes the albatross on Amsterdam Island is an allospecies in the superspecies *D. exulans*, that has adapted to subtropical conditions.

The total population including non-breeding adults and immature birds has been estimated at 30–50 individuals, and Amsterdam Island is certainly its only breeding site. Such a tiny and slow-breeding bird population is very susceptible to disturbance, so we would propose



The three largest representatives of the family *Diomedea*: the Wandering Albatross (*D. exulans*), the Royal Albatross (*D. epomophora*) and the new species (*D. amsterdamensis*).

that the breeding site (400 ha) be made a conservation area. □

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wide range for the unknown Higgs mass and the top-quark mass, there are strict limits on M_Z given M_w , regardless of the value taken for $\sin\theta_w$. The variation of M_Z with M_w was almost linear in this mass region and for a given M_w , M_Z was determined with error of less than 0.5 GeV. Sirlin stressed that it is preferable to eliminate θ_w altogether from this analysis, especially as $\sin\theta_w$ is determined in experiments where there are strong interaction corrections which are hard to quantify. He preferred to write down the direct relationship between M_w and M_Z in the standard model. This is

$$M_Z = M_w / (1 - (A / M_w)^2)^{1/2} \quad (4)$$

where A depends only on G (determined from the muon lifetime) and α . At tree level $A = 37.28$ GeV, but including radiative corrections $A = 38.66$ GeV.

The argument can also be reversed. From the experimental measurements of M_w and M_Z , A and hence the muon lifetime can be calculated; so if the standard model is correct, this procedure should be self-consistent. This is a very stringent requirement on the vector boson masses: for example, taking $M_w = 81$ GeV and $M_Z = 97$ GeV, a muon lifetime is

obtained which is about double its actual value. Using the central value $M_Z = 95$ GeV of the UA1 report¹¹ still gives a muon lifetime about 50 per cent too long.

The primary test then of the success of the standard model quantitatively lies in the precise determination of the masses of W and Z to better than 1 per cent to see whether they are consistent with the muon lifetime using equation (4). If not, the model can be patched up — another Higgs can be introduced for example — but advocates of the view that the standard model simply provides an effective theory rather than the basic quantum field theory of elementary particles will be on stronger ground. □

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Nonlinear dynamics

Simplicity and universality in the transition to chaos

from Alan Wolf

MANY systems undergo a transition to chaotic behaviour when subjected to increasing 'stress'. One such system, appealing because of its experimental simplicity, is a dripping faucet¹. With increasing flow rate, simple periodic dripping gives way to states of varying degrees of disorder, culminating in highly erratic motion. There have been three classical explanations for the origin of chaos: the irregular appearance of a signal containing many incommensurate frequencies; the nonlinear interaction of a large number of small simple systems; and the effect of ever-present environmental noise on otherwise regular motion. However, recent progress in the field of nonlinear dynamics demonstrates that simple deterministic mathematical models can explain several of the experimentally observed routes to chaos. Especially surprising are the predictions that certain features of chaos are 'universal', that is, largely independent of a system's detailed structure. The latest experimental confirmation of universality in chaos comes from studies of a Josephson-junction analogue by Yeh and Kao².

A particularly fruitful line of inquiry for nonlinear dynamics has been the study of one-dimensional maps of the unit interval $x_{n+1} = f_i(x_n)$, where the functions $f_i(x)$ have a single maximum in x and a tunable stress parameter λ . The 'logistic' equation of population dynamics, $x_{n+1} = \lambda x_n(1 - x_n)$, is one such map. Given a value of λ and a randomly chosen initial condition x_1 ,

an infinite sequence of numbers x_i (a discrete orbit) is generated. For sufficiently small stress, x_i tends to a constant value — a periodic orbit with period one. At larger stress there is a 'period-doubling bifurcation', a transition to a period-two orbit, where x_i takes on two values alternately. An infinite number of further period-doublings occur over successively smaller intervals in λ , terminating in an aperiodic orbit at finite λ . For still larger stress, a complex sequence of periodic and chaotic orbits is observed. Chaos is formally defined by the property that nearby orbits show a rapid (exponentially fast) divergence in their time evolution. In numerical simulations this 'sensitivity to initial conditions' is dramatic: if the initial conditions p_1 and q_1 differ by 1 part in 10^{12} , the action of a given chaotic map may cause p_{50} and q_{50} to differ by 1 part in 10.

The first evidence for universality in one-dimensional maps was found a decade ago by Metropolis, Stein and Stein³. They showed that the order of appearance of the various periodic states with increasing stress (denoted by the 'U-sequence') was, for a large class of maps, independent of the exact form of the map. In 1976 Feigenbaum discovered that one-dimensional map universality is more than qualitative in nature⁴. He observed that after many period-doublings, the intervals in λ between successive doublings shrink geometrically by the same factor, $\delta = 4.66920 \dots$, for all maps with quadratic maxima. Universal predictions were also

made for the spacing between elements of period-doubled orbits⁴, the effect of external noise on these states⁵, the character of intrinsic 'dynamical noise'⁶ (noise reflecting the aforementioned sensitivity to initial conditions) and other characteristics of chaos.

The physical relevance of this theory may not be readily apparent, as complex systems are not often explicitly governed by one-dimensional maps. In fact, although Lorenz⁷ discovered a one-dimensional map within a highly simplified model of convection as far back as 1963, only recently has the period-doubling route to chaos been discovered in such nonlinear systems as electrical circuits⁸, optically bistable cavities⁹, convecting fluids¹⁰ and the dripping faucet.

The manner in which a discrete map may lie hidden within a continuous system is illustrated in Fig. 1. Figure 1a shows a single long orbit describing the time evolution of a chaotic chemical reaction involving a large number of chemical intermediates. The state of the system at each instant is described by the concentrations of these chemical species, which provide the coordinates of a single point on the orbit. The successive intersections of the orbit with the dotted line provide us with a sequence of 'heights', x_i (essentially amplitudes of the chemical oscillations), which we may take to lie in the unit interval. In Fig. 1b a plot of the pairs (x_n, x_{n+1}) shows that each number predicts its successor, confirming that the random character of the chemical reaction is captured by a deterministic map. The U-sequence of periodic states was found in this experiment when a stress parameter, the rate at which additional chemicals were injected into the reaction cell, was varied¹¹.

As well as describing the period-doubling route to chaos, one-dimensional maps provide one explanation for 'inter-

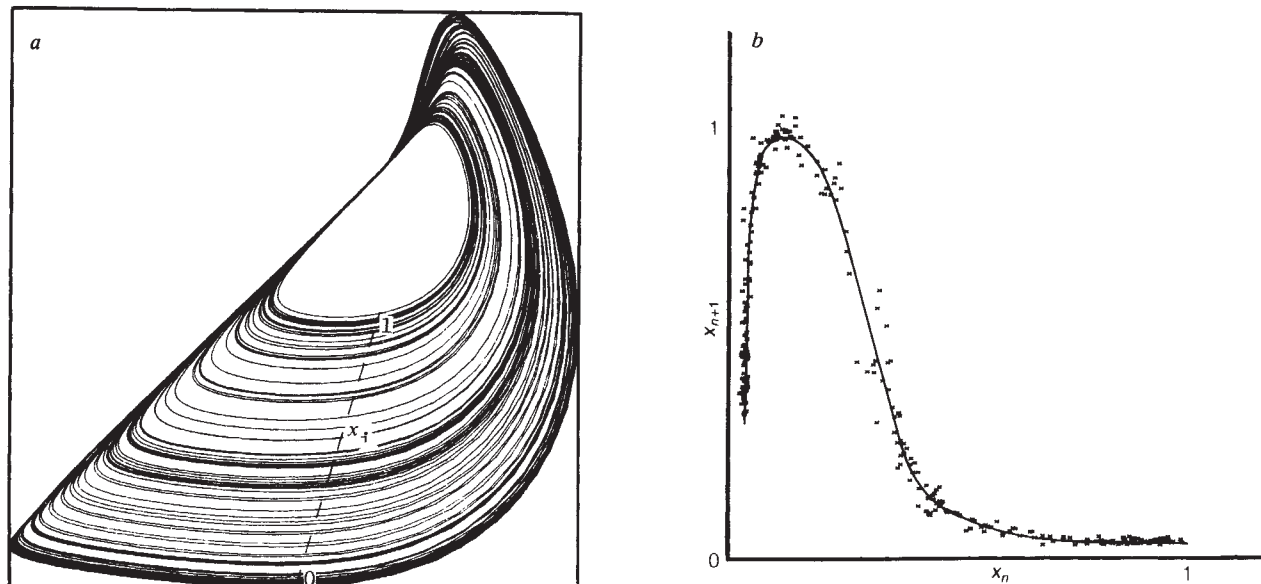


Fig. 1 a, Two-dimensional projection of experimental data¹¹ for a chaotic chemical reaction. The i th intersection of the orbit with the dotted line occurs at height x_i . b, A one-dimensional map is recovered from the chemical data by plotting the pairs (x_n, x_{n+1}) .

mittent' behaviour, in which long periods of regular motion are interrupted by occasional chaotic 'bursts'. This is illustrated in Fig. 2, which shows the voltage in Yeh and Kao's Josephson-junction analogue as a function of time. By varying the stress parameter, in this case the amplitude of the alternating current driving the circuit, the average interval between bursts can be set to any desired value. It is difficult to conceive of a deterministic origin for intermittency, yet Manneville and Pomeau discovered¹² that it is a simple property of the one-dimensional maps that govern systems such as this one. The implications for the analysis of experimental data are clear — what seems quite 'obviously' to be

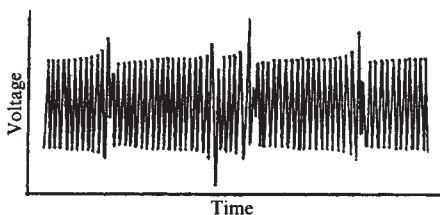


Fig. 2 Voltage as a function of time in a Josephson-junction analogue². Nearly periodic behaviour is interrupted by occasional chaotic 'bursts'.

stochastic behaviour may arise from deterministic dynamics.

Nature contains many far-from-equilibrium systems that are liable to exhibit chaos. Some of these will be understood through models whose complexity and universal character are comparable to those of one-dimensional maps. There are also chaotic phenomena, such as fully developed hydrodynamical turbulence, that are not likely to be understood by any simple mathematical model. Yet the fact that one-dimensional maps may be adequate to describe such diverse phenomena as cardiac dysrhythmia¹³ and chaos in the Mixmaster Universe¹⁴ suggests that the heart of chaos is mathematically accessible. The intriguing question that remains to be answered is, how much of our chaotic world is dominated by the simple universal varieties of chaos? □

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Nonlinear dynamics

Chaos in complicated systems

from Arun V. Holden

THE current entrancement with chaos¹ began with the realization that extremely simple nonlinear systems could, with appropriate values of parameters, have irregular complicated solutions. As discussed in the previous article, solutions often change from being periodic to being chaotic in a stereotyped way². Such powerful results, where complicated nonperiodic behaviour is produced in a simple way, lead to the hope that some of the irregularities of nature — the babbling of a brook or the mindless fluttering of a butterfly — may also be chaotic, and explicable by simple nonlinear systems with few degrees of freedom. But relating experimental observations to the chaotic limit sets of mathematical models is speculative. A tentative step is to identify and characterize irregular data as if they formed a sample solution of a deterministic system in a chaotic domain. Methods for doing this, and their applications, were considered at a recent workshop*.

An irregular experimental time series may be treated as if it were chaotic if it is possible to reconstruct an attractor of low fractal dimension from the data: this may be done graphically by plotting the delayed time series against itself³. Methods exist for estimating the fractal dimension of the reconstructed attractor⁴, and for estimating the rate of divergence of neighbouring trajectories⁵: it is not only possible to identify chaotic activity, but to measure how chaotic it is.

A different approach is to construct a simple nonlinear map that can be used to simulate some aspects of the experimental data: an example is to plot the peak amplitude of one oscillation against the peak amplitude of the preceding oscillation⁶. Another example is a Poincaré first return map, where the value a trajectory has on passing through a plane perpendicular to the trajectory is plotted against the value it has the next time it passes through that plane. These approaches are less demanding on computer time, and provide a working model, rather than quantitative measures, of chaotic activity.

Since chaotic solutions can be generated by simple nonlinear systems, it is natural to look for examples of chaotic activity in simple physical systems such as a dripping faucet. It is also possible to look for chaotic activity in complicated systems — the most complicated systems we know are living.

The heart may be considered to act as a nonlinear oscillator, so perhaps some cardiac dysfunctions correspond to chaotic behaviour⁷. Glass *et al.*⁸ have examined the response of endogenously active aggregates of embryonic cardiac muscle cells to periodic stimulation. An accurate

description of such an aggregate is complicated, as not only are there many voltage-dependent conductances in cardiac membrane, but there is also the probability of activity-dependent changes in the ionic concentrations in restricted extracellular spaces. However, the dynamic behaviour during repetitive stimulation is simple — as the frequency is increased there is a sequence of period-doubling bifurcations into a region of aperiodic chaotic activity. The experimentally determined Poincaré return maps may be iterated, to simulate the response to periodic stimulation, or since the bifurcation sequence for single-peaked one-parameter maps of an interval into itself is largely independent of the detailed form of the map, a simplified caricature of the map can be analysed. Thus a periodically driven complicated system that exhibits chaotic activity can be represented by a simple nonlinear mapping.

Periodically excited nerve cells can also show chaotic behaviour⁹ — what is of more interest is whether neurones¹⁰ or neural systems can generate chaotic activity endogenously. Such chaotic activity might be pathological, or might form part of the normal irregular background activity of the nervous system. Methods now exist for investigating these possibilities.

Although no manufactured system approaches the complexity of biological systems, large-scale integration techniques have produced a generation of high-density complicated electronic control systems. Such computing devices are deterministic finite-state automata, and so it is natural to assume that their behaviour is fully predictable. Any irregularity in performance is usually ascribed to poor programming or to component failure. But complicated as well as simple nonlinear systems may exhibit chaotic solutions. Chaotic activity in a laboratory computer system may be inconvenient; in a locally autonomous complicated military system it could be unfortunate¹¹. One can only hope that as the complexity of a system increases, access to any domains of chaotic activity becomes more and more unlikely. □

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Plant biochemistry

Phytochrome feedback

from G.C. Whitelam

PHYTOCHROME is the photoreceptor which regulates almost all aspects of plant development including germination, seedling development, flowering and adaptation to the light environment. The molecular mechanisms underlying its action have yet to be elucidated but several examples of phytochrome-regulated gene expression have been recognized¹⁻³. A particularly intriguing example was described recently: phytochrome exerts autoregulatory control over its own translatable mRNA level. The finding was one of a number of significant new insights into the nature of the phytochrome molecule to be reported at the European Symposium on Photomorphogenesis in Plants*.

Phytochrome is a soluble protein existing in two forms — a red-light-absorbing form (Pr, $\lambda_{\text{max}} = 666 \text{ nm}$) and a far-red-light-absorbing form (Pfr, $\lambda_{\text{max}} = 730 \text{ nm}$) — which are converted into one another by light of suitable frequency. Photoconversion of Pr to Pfr induces a diverse array of morphogenic responses, and induction is halted when Pfr is converted back to Pr. Pfr is thus often considered to be the active form and Pr the inactive form of phytochrome.

It has been held that phytochrome is synthesized *de novo* as Pr at a constant rate and accumulates in dark-grown plant tissue. The rapid decline in phytochrome concentration seen on photoconversion of Pr to Pfr has been believed to be the result of the very much greater rate of Pfr degradation relative to Pr. The low steady-state concentration of phytochrome in continuous light, characteristically 1–3 per cent of the initial dark-grown tissue level, has thus been accounted for solely in terms of the differential turnover rates of Pr and Pfr: a slower rate of Pr degradation and a more rapid rate of Pfr degradation against a background of constant Pr synthesis.

New work reported from Peter Quail's group in Madison, Wisconsin now clearly shows that there is another level of control. In a rabbit reticulocyte lysate *in vitro* translation system, the translatable phytochrome mRNA accounts for only about 0.005 per cent of the poly(A) RNA from dark-grown oat shoots. As little as five seconds' exposure to red light induces a marked and rapid reduction in the level after about 15 min and a 95 per cent reduction of phytochrome mRNA after only 2 h. Control experiments show that this represents a selective decrease in translatable phytochrome mRNA. Partial reversal of the red-light-induced decline by subsequent far-red light indicates that phytochrome itself is the photoreceptor.

Only partial reversibility is possible since the 1 per cent Pfr level established by far-red light alone is sufficient to induce a significant response. As Pfr becomes depleted in extended periods of darkness the feedback control appears to be reversed since translatable phytochrome mRNA reaccumulates. The rapidity of the regulation, with a lag of only 15 min, makes this one of the most promising systems for the elucidation of the transduction chain between receptor and gene expression.

An obvious consequence of the demonstration of the autoregulatory control mechanism is that current concepts regarding the control of phytochrome levels *in vivo* will have to be revised. Indeed, introduction of the second level of control has already created something of a problem. It is known that the rate of phytochrome degradation is increased by up to 100-fold in the light and we now know that its rate of synthesis decreases by about 20-fold in these conditions. This being the case, then the levels of phytochrome present in continuous light should be an order of magnitude lower than observed.

Of the possible explanations put forward to account for the discrepancy, one which has created most interest, as well as obtaining some experimental support, is the long-established notion that there may be two distinct populations of phytochrome. The major population, with a high Pfr destruction rate, would be rapidly depleted in the light, leaving a minor more stable population to predominate. Recent experimental evidence on the kinetics of Pfr degradation in *Amaranthus* supports this suggestion⁴.

One view is that the two populations represent distinct gene products with different immunological properties as well as distinct turnover rates. Support for this notion was presented by Brian Thomas (Glasshouse Crops Research Institute, Littlehampton) who described the development of an enzyme-linked immunosorbent assay for phytochrome using antibodies raised against phytochrome from dark-grown oats. The results of immunotitration experiments revealed that the avidity of the antigen-antibody reaction for phytochrome from dark-grown oat shoots was much higher than that for phytochrome from light-grown tissue. Independently, Quail's group has also reported these immunological differences based on the results of immunoprecipitation experiments. The inability of antibodies to phytochrome from dark-grown tissue to precipitate effectively phytochrome from light-grown tissue explains the failure to detect a phytochrome translation product among the total *in*

vitro translation products encoded by poly(A) RNA from light-grown tissue^{5,6}.

The existence of two different phytochromes seems a real possibility then, although more trivial explanations such as post-homogenization modifications do have to be considered. New techniques may soon provide a firm conclusion. Quail described the production of a cDNA probe for the mRNA for dark-grown oat phytochrome, which should prove an invaluable tool in elucidating further the molecular biology of phytochrome; it will be interesting to see whether there is indeed a second phytochrome gene. Rick Vierstra (University of Wisconsin, Madison) and Clark Lagarias (University of California, Davis) both described new purification protocols for production of proteolytically undenatured phytochrome from dark-grown tissues. However, no one has so far succeeded in the daunting task of purifying phytochrome from green tissue. A number of groups are currently producing monoclonal antibodies to phytochrome. M-M. Cordonnier (University of Geneva) described work carried out jointly with L. Pratt (University of Athens, Georgia) on the characterization of monoclonal antibodies to oat and pea phytochromes. Interest is currently focused on the development of clones which will be specific for Pr and Pfr and on the characterization of clones exhibiting extensive cross-reactivity in the expectation that cognate antigenic sites represent highly conserved regions of the phytochrome molecule and so may be involved in its biological activity. □

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100 years ago

PROF. BROWN GOODE, the Commissioner of the United States to the International Fisheries Exhibition, has just received a telegram from Prof. Baird, the United States Commissioner of Fish and Fisheries, to the effect that Mr. Ryder, the embryologist of the Fish Commission, has finally solved the problem of the culture of oysters from artificially impregnated eggs, and that on the 4th inst., at the Government station at Stockton, Maryland, there were many millions of young oysters three-quarters of an inch in diameter which had been hatched from eggs artificially impregnated forty-six days before. It may be added that oysters were artificially impregnated in America by Dr. Brooks, of Baltimore, in 1879, but the difficulty hitherto met with in hatching them has been to prevent the young oysters from escaping and being lost immediately after they are hatched, since the spat passes through the meshes of most finely-woven fabrics, such as flannel. From *Nature* **28**, 470, 13 September 1883.

*The symposium was held in Frostvallen, Sweden on 19–23 July 1983.

Rare earth elements in seawater near hydrothermal vents

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Rare earth element (REE) patterns in the deep Pacific are strongly depleted in the lighter elements and have a large negative cerium anomaly. These REE patterns and associated concentration–depth profiles are maintained by regeneration in deep waters modified by preferential scavenging of the lighter elements. Scavenging by iron- and manganese-rich hydrothermal plumes might explain why vast areas of sediments far removed from spreading centres contain a metalliferous component.

EARTH scientists have long been intrigued with the rare earth elements (REE). The REE form a coherent group because of their systematic decrease in atomic radii with increasing atomic number and predominant +3 oxidation state. The fact that two members of the group, cerium and europium, are often found in anomalous oxidation states only adds to the group's appeal. Oceanographers first measured the seawater concentrations of these elements in the 1960s when levels in the pmol kg^{-1} range were reported^{1–3}; these findings have been supported by recent work^{4–7}. If rare earth concentrations in the deep sea are normalized to shale⁸ and plotted against atomic number, a striking pattern emerges: seawater is strongly depleted in the light (LREE) (that is, La–Eu). If we assume that shale is representative of the material brought to the oceans by rivers then this pattern implies that there is some process preferentially removing the LREE from estuaries/seawater. The data presented here help us to understand the nature of this process and thus what determines the REE patterns of seawater. These data also represent the first detailed concentration–depth REE profiles reported for the Pacific Ocean and demonstrate effects of hydrothermal activity on the geochemistry of these elements in the oceans.

Our laboratory combines clean-chemistry techniques with isotope dilution mass spectroscopy to overcome problems of contamination and make it possible to analyse 500–2,000-ml samples for nine REE. Working with small sample volumes leads to analytical complications involving isobaric interferences and small ion beams which makes it difficult to determine all nine elements in every sample. In addition, the precisions of the REE data here vary from $\pm 8\%$ (2σ) for Ce to $\pm 3\%$ for Nd which are worse than precisions obtained with larger samples⁹. Still, these uncertainties are well within the natural variability of these elements in the oceans.

Hydrothermal sites

Samples were collected during two cruises in 1981 to hydrothermal sites in the Pacific Ocean. Rama 10 was a geophysical/geochemical survey of the spreading centre in the Mariana Trough (18°N , 145°E). This crescent-shaped back-arc system has an opening rate of $0\text{--}8 \text{ cm yr}^{-1}$ (ref. 10) and an active hydrothermal convection system which has been documented by high heat flow and metalliferous sedimentation¹¹ as well as

manganese and methane plumes (K. Kim, personal communication) above the sea floor. Some of the samples for this study were collected from a transect of stations across the spreading centre at 18°N but most hydrocasts were concentrated over a mounds field some 60 km west of the main extrusion zone. Samples from the East Pacific Rise (EPR) were collected during the eighth leg of the Vulcan Expedition which included Sea-beam sonar mapping and Angus photographic mapping of an ultra-fast (opening rate of $16\text{--}18 \text{ cm yr}^{-1}$) section at 19°S . Two major hydrothermal fields were discovered during Vulcan 8 and the associated plumes were sampled for methane, helium, manganese and REE.

Manganese is a sensitive tracer of plume dispersion¹² as the hydrothermal end member is a million times enriched relative to ambient seawater and resists oxidation for some time after being discharged with vent effluents. Samples collected from plumes at Vulcan 8 stations positioned directly over the spreading axis of the EPR at 19°S contained 15 times more hydrothermal manganese than the 'hottest' sample from the Mariana Trough; that is, the maximum manganese concentration observed at the Mariana was 4 nmol kg^{-1} whereas plume samples from the EPR contained as much as 60 nmol kg^{-1} . This is not to say that the influence of hydrothermal activity on seawater chemistry is greater over the EPR but simply that this particular suite of samples obtained from the Mariana Trough was more diluted with ambient seawater.

Results

Data from eight stations occupied during Rama 10 are displayed in Fig. 1. We averaged these results from the Mariana Trough for each depth and superimposed the curves on data from the EPR (Fig. 2). This comparison clearly shows that REE concentrations are lower in the South Pacific while the trends with depth are similar. The surface water concentrations in the South Pacific are the lowest REE concentrations yet observed in the oceans. While surface water levels in the North Pacific are higher, they are still two to six times lower than those observed in the North Atlantic (Table 1). Since the input of ^{210}Pb (ref. 13) and total lead¹⁴ to the surface ocean from the deposition of atmospheric particulates is at least three times lower in the North Pacific than in the North Atlantic, the difference in REE levels between these locations is consistent with a major aeolian source for the REE as postulated by Elderfield and Greaves⁵.

No systematic differences in REE profiles from the Mariana Trough were found with respect to latitude, longitude or

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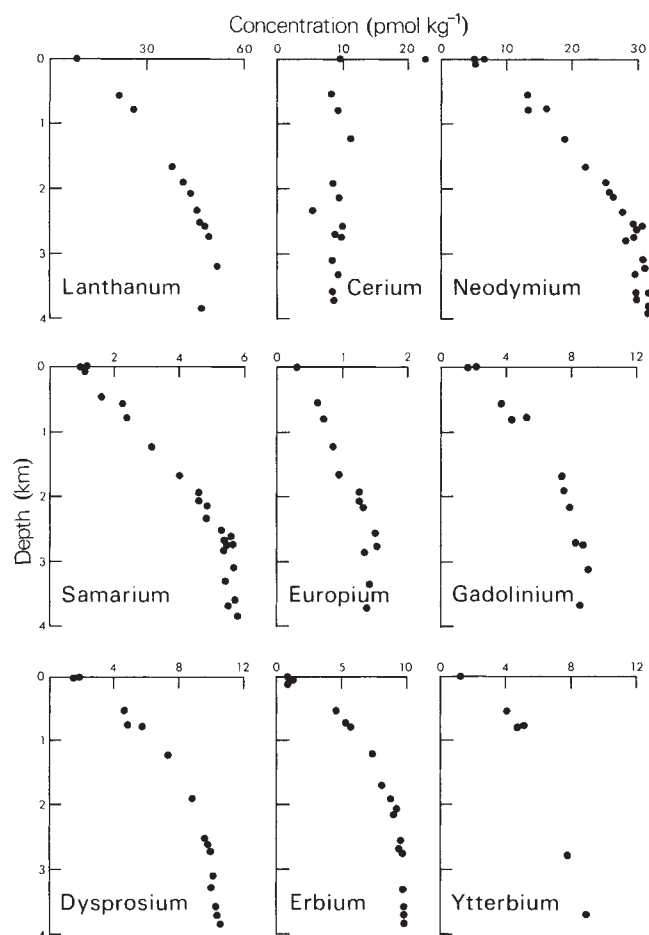


Fig. 1 REE concentration–depth profiles in the Mariana Trough.

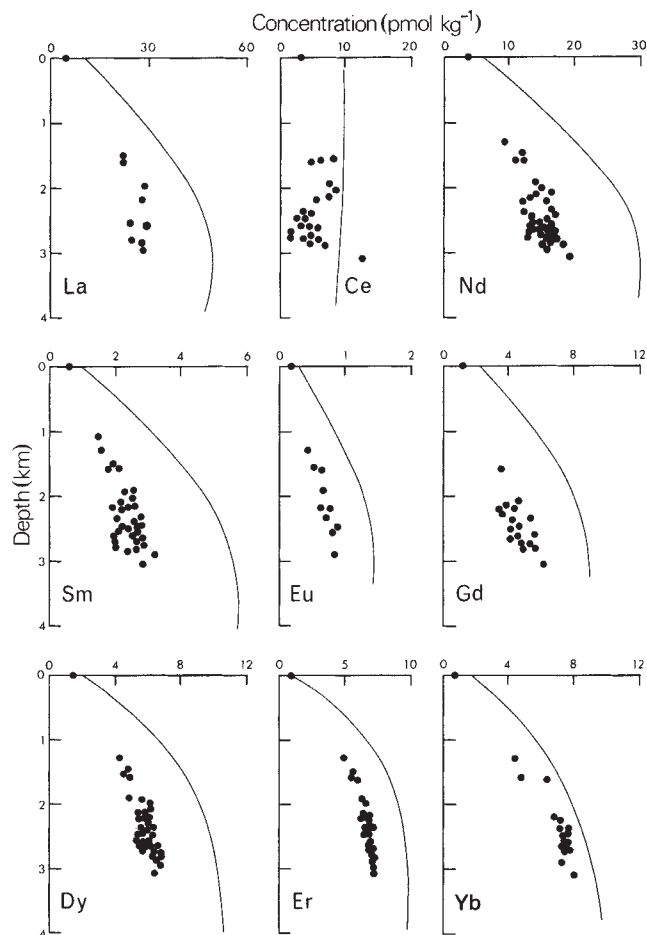


Fig. 2 REE concentration–depth profiles from the South Pacific. The curves represent data from the Mariana Trough (Fig. 1) averaged at each depth.

proximity to the spreading centre. Eight of these elements have profiles which show increasing concentrations with depth; cerium is anomalous and generally decreases with depth. The cerium data from the EPR are scattered with no apparent trend while the other elements increase with depth similar to the Mariana data but with two important differences: the EPR data are more variable and the concentrations are generally lower. This variability is real as Nd and Sm from the EPR are as strongly correlated as at the Mariana (Fig. 3b) and display a similar relationship at the two sites. On the other hand, Er covaries linearly with silica in the Mariana Trough (Fig. 3a) but is not simply related to Nd or Sm at either site (Fig. 3c). Several geochemical processes could fractionate LREE and HREE in this way but the following evidence suggests that preferential scavenging of the lighter elements is important.

Geochemical evidence

Four processes could affect REE concentration–depth profiles at these hydrothermal sites:

(1) The HREE could be more affected than the LREE by a deep-regeneration cycle similar to silica. The observations that HREE levels in deep waters increase systematically from North Atlantic to North Pacific (Table 1) and that HREE concentrations and silica are correlated (for example, Er; Fig. 3a) suggest that a nutrient-like regeneration process is responsible for the general shape of their profiles and the variability between basins. However, the REE are not simply cycled with nutrients as the Er–Si relationship varies with location (Fig. 3a). Moreover, their concentrations can vary in waters having identical nutrient levels¹⁵ as we observe in the South Pacific (Fig. 4a).

Table 1 REE seawater concentrations (pmol kg^{−1}) at various locations and two depths

	NE Atlantic ⁵		NW Atlantic ^{7*}		SE Pacific		NW Pacific	
	Surface	2,500 m	Surface	2,486 m	Surface	2,500 m	Surface	2,500 m
La	36.7	29.4	15.0	36	4.9	30	8.3	47
Ce	66.3	26.1	86	17	3.1	3.5	10	9.0
Nd	34.3	25.0	—	—	3.4	16	5.1	30
Sm	6.01	4.75	3.7	3.3	0.56	2.7	1.0	5.3
Eu	0.615	0.895	0.78	0.72	0.20	0.80	0.33	1.4
Gd	5.59	7.19	—	—	1.1	5.0	1.6	8.2
Dy	5.00	6.10	—	—	1.3	6.3	2.0	9.7
Er	3.63	5.09	—	—	1.2	7.0	1.7	9.4
Yb	3.15	4.79	4.3	5.0	0.79	7.5	1.1	8.0

* Only 5 of the 10 REE measured by De Baar *et al.*⁷ are reported here. La and Ce concentrations were interpolated from their tabulation.

(2) The truncated profiles in bottom waters of the Mariana Trough could be an artefact of circulation over a 2,800–2,900-m sill. The presence of the sill is quite apparent from silica and temperature data across the region (Y. Horibe, unpublished data) but its effect on these profiles is unclear. The EPR results suggest another process is more important as deep waters at stations on and off the rise crest at 19° S are of the same water mass¹⁵ but have different REE levels (Fig. 4a).

(3) REE levels near vents might be expected to be anomalously high if REE concentrations in vent fluids were high enough. Two observations suggest that direct injection with vent fluids is unimportant. First, measurements by Michard *et al.*¹⁶ of REE in vent fluids from the EPR at 13° N suggest that these levels are too low (with the possible exception of europium) to affect bottom water concentrations. Second, if direct injection of REE with hydrothermal effluents was the most important process, we would expect to see higher levels in the EPR samples which contained more hydrothermal manganese and, in fact, we see just the opposite (Fig. 2).

(4) Scavenging of REE by oxidation products of the manganese and iron discharged with hydrothermal effluents may be affecting these profiles. If the lighter elements are preferentially scavenged, this mechanism would explain the relationships demonstrated in Fig. 3. We favour this explanation, which is supported by several lines of evidence. First, REE patterns from the Mariana Trough become more LREE-depleted with increasing depth and the anomaly of cerium, which is readily oxidized to +4 and incorporated into manganese oxides^{2,17}, becomes increasingly negative with depth (Fig. 5a). Neither of these trends is observed in the deep North Atlantic^{5,7} where the cerium anomaly is also negative but much smaller (Fig. 5b). Second, the trends of the EPR data in Fig. 4a suggest that

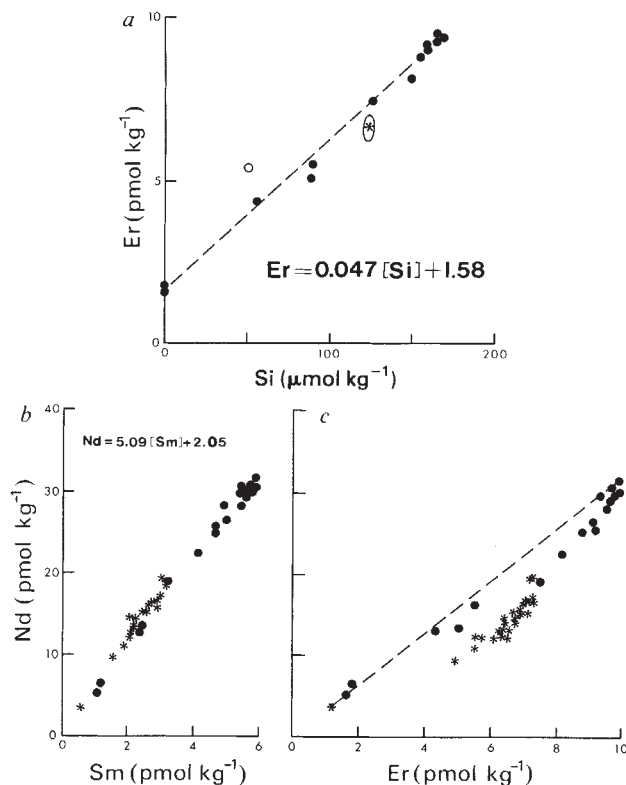


Fig. 3 ●, Mariana Trough; ○, North Atlantic⁵; *, South Pacific. a, Er versus Si. Dashed line, a linear best-fit described by the equation. b, Nd versus Sm with best-fit equation. c, Nd versus Er. The dashed line connects surface and bottom waters.

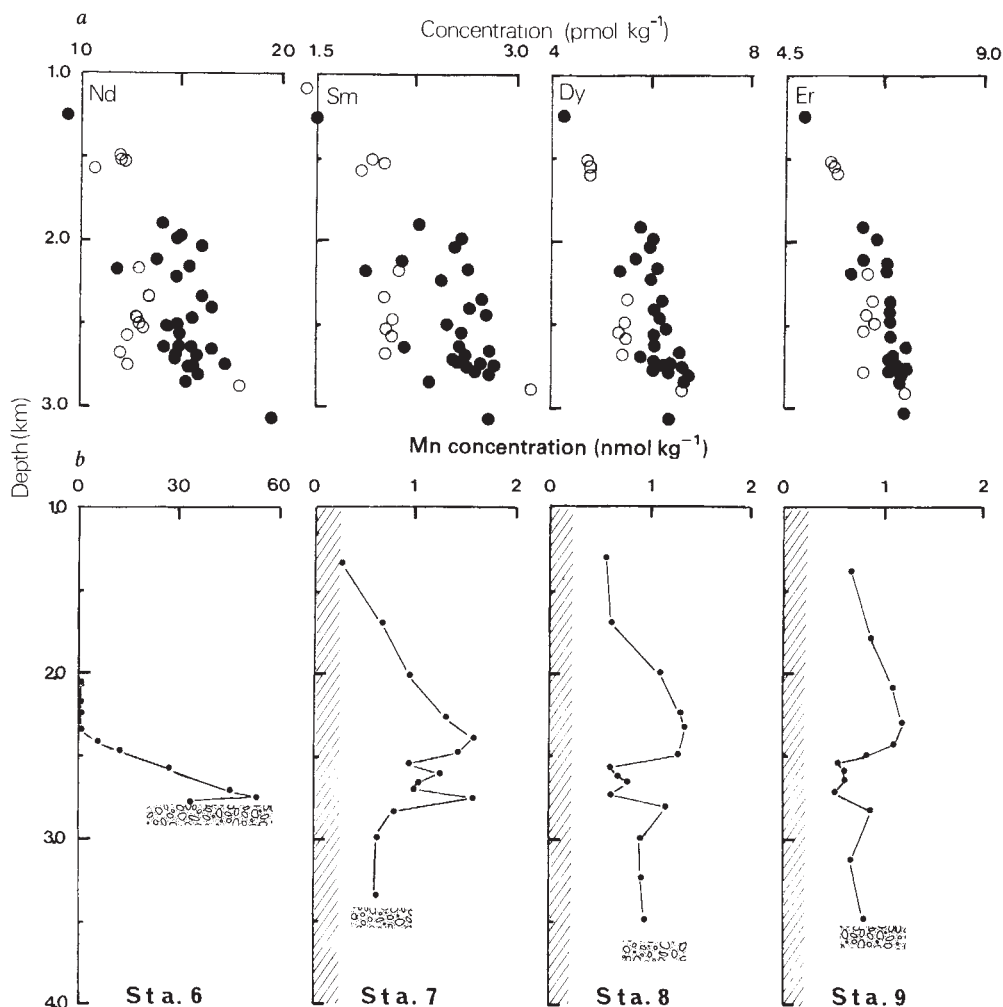


Fig. 4 a, REE concentration-depth profiles from the South Pacific. The open symbols were collected at off-axis stations and the solid symbols at on-axis stations. b, Manganese-depth profiles moving west from the axis of the EPR at 19° S; station 6, on the axis; station 7, 330 km; station 8, 750 km; station 9, 1,400 km.

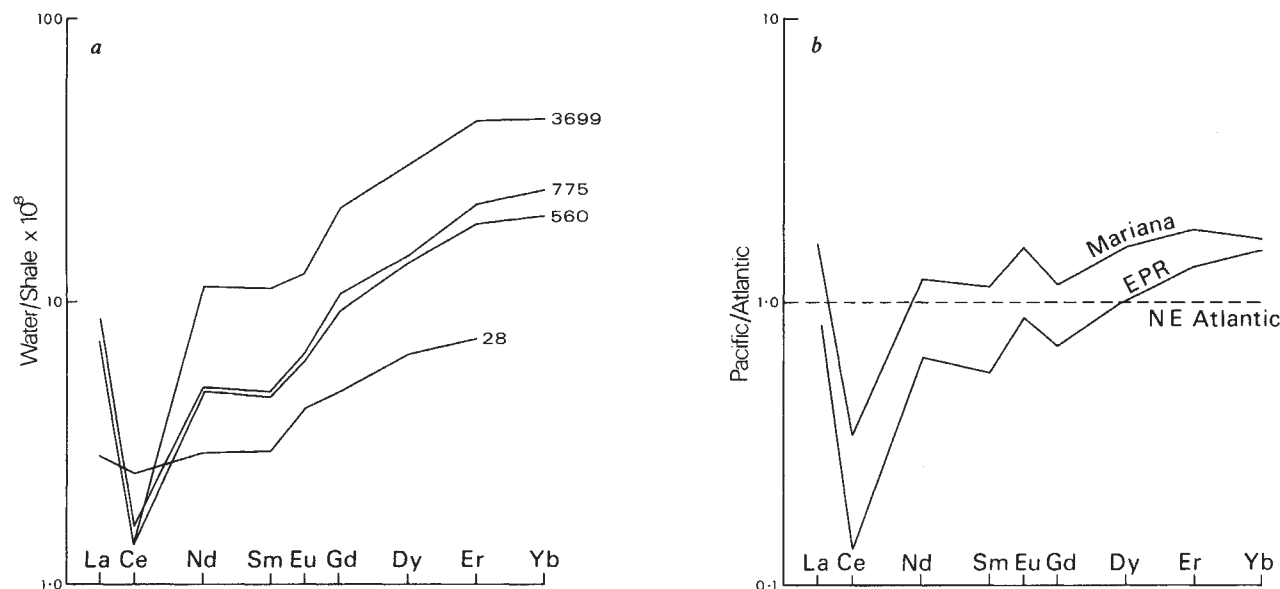


Fig. 5 *a*, REE concentrations for the Mariana Trough normalized to shale⁸ and plotted versus atomic number. The number to the right of each pattern is the depth in metres. *b*, REE concentrations in the Pacific normalized to those in the North Atlantic⁵. All samples were collected from 2,500 m.

concentrations below 2 km at off-axis stations are anomalously low and not that on-axis stations are anomalously high. Third, there is direct evidence that scavenging by hydrothermal oxidation products is plausible as manganese anomalies of $\sim 1 \text{ nmol kg}^{-1}$ persist below 2 km even at locations which are 300–1,400 km away from vent fields (Fig. 4b). While these off-axis manganese profiles are complex and surely represent an integrated signal from several hydrothermal sources, they are systematic and thus establish a link with hydrothermal activity on the rise crest. Filtering demonstrated that 90% of the manganese in the plume at these off-axis stations is $< 0.4 \mu\text{m}$. Oxidation of this manganese would provide fresh surfaces for scavenging. It is also possible that fine-grained iron colloids formed near vent fields and transported in the plume could also be important scavengers at these remote locations as they certainly are nearer the vents¹⁸. The intrusion of a hydrothermal plume to these longitudes has also been documented using contours of $\delta(^3\text{He})$ (ref. 19). These manganese and helium anomalies suggest a westward advection of the plume at 19°S in agreement with the latest circulation model¹⁵ and the asymmetrical distribution of metalliferous sediments at this latitude^{20,21} as first pointed out by Edmond *et al.*¹⁸. Fourth, differences between on and off-axis stations disappear above 2 km and the variability of data below this depth decreases systematically with increasing atomic number (Fig. 4a), suggesting a greater degree of scavenging in the plume with the LREE being the more reactive.

The evidence presented here for discharge, dispersion and scavenging from the water column is supported by REE patterns

in metalliferous sediments²² which indicate a seawater source for these elements and the observation that sediment in the South Pacific far removed from the EPR can contain a metalliferous component^{20,21}. These Mn and REE results suggest that vast areas of the oceans are affected by hydrothermal activity; a conclusion consistent with our observation that $< 1\%$ of the hydrothermal manganese in waters of the South Pacific resides over the EPR. We can only guess how diffuse this process is, but the overall effect of scavenging by hydrothermal products on REE geochemistry can be demonstrated by normalizing the results from 2,500 m at these vent sites to data from the North Atlantic⁵ for the same depth (Fig. 5b). This normalization suggests that the dominant effect of hydrothermal circulation on REE geochemistry in seawater is preferential removal of the lighter elements. This pattern is enhanced by more efficient regeneration of the heavier elements in deep waters, that is, the HREE have longer residence times⁵. This logic is consistent with the chemistry of these elements which predicts that the HREE should form the more stable complexes in seawater² and thus be less susceptible to scavenging. Since precipitation of manganese/iron oxides is ubiquitous in natural waters, fractionation during the scavenging process may well fix the REE patterns of seawater.

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Construction of artificial chromosomes in yeast

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Fifty-five-kilobase long artificial chromosomes containing cloned genes, replicators, centromeres and telomeres have been constructed in yeast. These molecules have many of the properties of natural yeast chromosomes. Centromere function is impaired on short (less than 20 kilobases) artificial chromosomes.

CONTINUING attempts have been made to relate the behaviour of chromosomes in mitotic and meiotic cell cycles to their structure. These studies have largely been performed by manipulation of naturally occurring chromosomes by classical genetic techniques¹⁻³. The availability of recombinant DNA technology allows an alternative approach: the construction of artificial chromosomes from cloned fragments of DNA. We have used this strategy to investigate the structural requirements for normal chromosome function in the yeast *Saccharomyces cerevisiae*.

Four different classes of functional elements have been identified on natural chromosomes: genes⁴, replication origins^{5,6}, centromeres^{7,8} and telomeres⁹.

Stinchcomb, Struhl and Davis⁵ and Carbon and Hsiao⁶ identified sequences in the yeast genome which conferred on plasmids the ability to replicate extrachromosomally. These *ARS* (autonomously replicating sequence) elements are presumed to be chromosomal replication origins although there is no proof for this assertion. Plasmids which contain *ARS* elements are mitotically unstable and are lost rapidly when selective pressure is relaxed^{5,6}. This observation allowed the identification of functional yeast centromeres (abbreviated *CEN*) as sequences which increased the mitotic stability of *ARS*-bearing plasmids^{7,8}. Mitotic stability has been measured in three ways: (1) by determining the fraction of plasmid-bearing cells in selectively grown cultures^{5,6}, (2) by measuring the rate at which the plasmid is lost following the relaxation of selective pressure¹⁰, or (3) by pedigree analysis in which individual cell lineages are followed and plasmid loss is detected as the inability of single cells to give rise to colonies on selective medium¹¹. Pedigree analysis allows the direct determination of the segregation frequency, which is defined as the fraction of cell divisions (of plasmid-bearing cells) in which only one of the progeny cells receives the plasmid. For circular *ARS* plasmids 5–20% of selectively grown cells contain the plasmid^{5,6}, and the segregation frequency ranges from 0.3 to 0.6 (ref. 11). The addition of a centromere increases the mitotic stability such that the fraction of plasmid-bearing cells is 90% and the segregation frequency 0.01 (refs 7, 8, 10). In addition, the presence of a centromere causes the copy number to decrease from 20–50 to 1–2 plasmids per cell^{7,8}.

The ends of chromosomes are specialized structures known as telomeres. Analysis of linear DNA plasmids from the macronuclei of protozoans has shown that their termini consist of a tandem array of a simple repetitive sequence¹². These sequences function as telomeres on linear plasmids in yeast⁹. Using such plasmids Szostak and Blackburn⁹ have cloned a yeast telomere whose structure seems to resemble that of the *Tetrahymena* ribosomal DNA end. For technical reasons we have used the *Tetrahymena* rDNA termini (which we abbreviate Tr ends) rather than yeast telomeres in our experiments. The Tr ends we have used also function as *ARS* elements in yeast¹³.

Our approach has been to construct linear centromeric plasmids containing various combinations of cloned chromosomal elements, and to compare their behaviour with that of natural

yeast chromosomes. Their mitotic properties have been analysed by measuring plasmid copy number and mitotic stability, while we have used tetrad analysis to follow their behaviour in meiosis. Natural chromosomes are lost mitotically at a frequency of 10^{-4} to 10^{-5} (refs 14–16) and are maintained at one copy per cell in haploid strains. We find that centromeric linear plasmids between 7 and 15 kilobases (kb) long are present in many copies per cell and are much less mitotically stable than are circular *ARS* centromeric plasmids. In contrast, 55-kb centromeric linear plasmids show a high level of mitotic stability, are maintained at low copy number and behave meiotically like natural chromosomes.

Short centromeric linear plasmids

The construction of yeast linear plasmids is complicated by their inability to replicate in *Escherichia coli*. Our approach has been to construct circular plasmids in *E. coli* which contain yeast genes, replication origins and centromeres. We then add sequences that will function as telomeres by ligation *in vitro*¹⁷, transform the resulting ligation mix into yeast and screen the transformants to find those which harbour plasmids of the predicted structure. Figure 1 illustrates this method as applied to the construction of the linear plasmid YLp4. The circular plasmid A75p9 was constructed by standard recombinant DNA techniques and contains the *LEU2* gene, *CEN3* and *ARS1* in the cloning vector pBR322. The Tr ends were added by ligating an inverted repeat of the terminal 0.7 kb of the *Tetrahymena* rDNA plasmid into the single *SalI* site of A75p9. Previous experiments have shown that this inverted repeat resolves *in vivo*, converting circular molecules which bear it into linear plasmids having two stable ends¹⁷. After transformation of the ligation mix into yeast, Leu⁺ transformants were isolated, DNA was prepared from them and the plasmids they contained were mapped with restriction enzymes by Southern blotting¹⁸. The transformants TA708 and TA712 contain a linear plasmid of 10.7 kb whose restriction map is as predicted from those of its constituent elements (data not shown).

The mitotic stability of the linear plasmid YLp4 was measured by determining the fraction of plasmid-bearing cells. As shown in Table 1, the mitotic stability of YLp4 is much lower than that of the circular *ARS1*, *CEN3* plasmid A75p9, from which it was derived. YLp4 is no more stable than the acentric linear plasmid YLp30, which is identical to YLp4 except that it lacks the *CEN3* fragment.

To measure the copy number of circular and linear centromeric plasmids, we ran *HpaI*-digested DNA on an agarose gel and Southern-blotted it using radiolabelled DNA from the plasmid pSZ57 (ref. 19), which contains the *LEU2* gene and pBR322, as probe. The copy number was assessed from the intensity of the hybridization to the fragment containing the chromosomal *LEU2* gene relative to the *LEU2*-containing fragment from the plasmid. In the strain containing A75p9, the intensities of the chromosomal and plasmid-derived *LEU2* bands are equal, confirming the observation that circular *ARS*, *CEN* plasmids are maintained at one copy per cell. The band derived from YLp4 hybridizes much more intensely than the

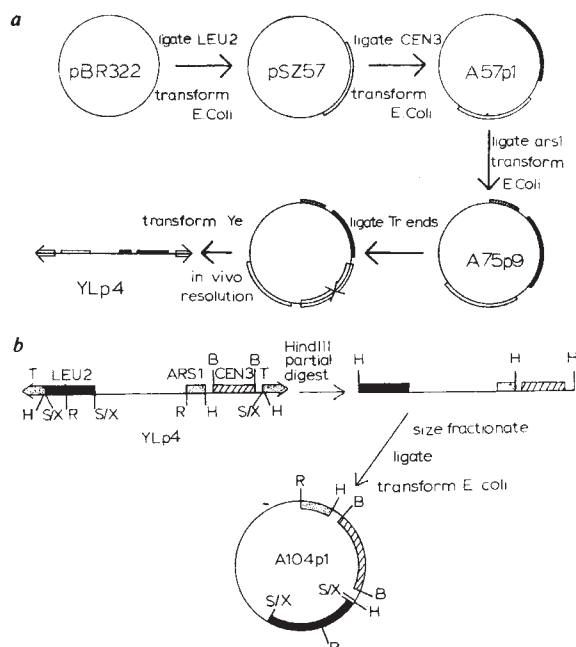


Fig. 1 *a*, Construction of the yeast linear plasmid YLp4. *b*, Recovery of a *CEN3*-containing circular plasmid from YLp4. Restriction enzyme sites: H, *HindIII*; S/X, *SalI*-*XhoI* junction; R, *EcoRI*; B, *BamHI*.

Methods: *a*, The construction of the *LEU2* plasmid pSZ57 has been described elsewhere¹⁹. pSZ57 was digested with *BamHI* and ligated to a gel-purified 1.9-kb *BamHI* *CEN3* fragment derived from the plasmid YCp82 (J. Hicks, personal communication). We identified A57p1 among the ampicillin-resistant transformants from this ligation mix by screening rapid DNA preparations by restriction enzyme digestion³⁰. A75p9 was made by ligating A57p1 cleaved with *PvuI* and *HindIII* to A71p4 (a pBR322 derivative containing *ARS1*, *LEU2* and the *TCM1* gene (A. W. M., unpublished)) cut with the same enzymes. The inverted Tr end repeat was prepared by gel-purifying a 0.7-kb *XhoI*-*HhaI* fragment containing the terminal sequences of the *Tetrahymena* rDNA and 14 bp of pBR322, derived from the plasmid pSZ222 (ref. 17), ligating this to itself and then recutting with *XhoI* after inactivation of the T4 DNA ligase. This head-to-head dimer was ligated to *SalI*-cut A75p9 in the presence of both *SalI* and *XhoI* in order to drive the reaction in favour of the desired product. The ligation mix was transformed into D234.3B with selection for *Leu*⁺ transformants. Plasmid mapping was performed on Southern blots of restriction enzyme-cleaved DNA. *b*, DNA was size-fractionated on a 0.5% agarose gel and recovered by electrophoresis into and subsequent high salt elution from DEAE paper. The structure of A104p1 and three other independent isolates was verified by restriction enzyme mapping DNA prepared from *E. coli*. Note that the *HindIII* site, in A104p2, adjacent to the *LEU2* gene, is derived from a site within the Tr ends and therefore is absent from A75p9. This excludes the possibility that the recovered circular plasmids arose from contamination of DNA preparations with A75p9.

chromosomal *LEU2* gene. We estimate that the copy number of this linear centromeric plasmid is about 15 copies per plasmid-bearing cell. For the acentric linear plasmid YLp30, the plasmid copy number is even higher (Fig. 2, lane 4). Although the presence of a centromere does decrease the copy number of linear plasmids and may slightly increase their mitotic stability, both of these effects are much weaker than those seen on the addition of a centromere to circular *ARS* plasmids. Recently, similar findings have been reported for linear plasmids carrying *CEN4* (ref. 20).

Although the restriction map of YLp4 was as predicted, we were concerned that the *CEN3* sequence might have suffered some mutation which reduced its ability to function as a centromere. We reconstructed a circular *CEN* plasmid from YLp4 using a *HindIII* site within the Tr ends (Fig. 1*b*). DNA from TA708 was partially digested with *HindIII* and fragments

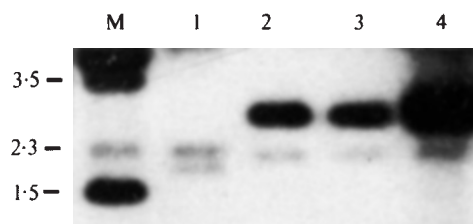


Fig. 2 Copy number of short linear plasmids. Lane M, molecular weight markers; 1, *HpaI*-*BamHI*-cleaved DNA from strain A281 carrying A75p9; 2, 3, *HpaI*-cut DNA from strain A281 carrying YLp4; 4, *HpaI*-cut DNA from strain A281 carrying YLp30. The chromosomal *LEU2* band is the upper band in lane 1 and the lower band in lanes 2-4. Molecular weights are shown in the left-hand margin.

Methods: DNA was run a 0.7% agarose gel, blotted according to Southern¹⁸ and hybridized to nick-translated pSZ57¹⁹ (pBR322 plus *LEU2*). The plasmid-derived bands which hybridize to pBR322 sequences are all greater than 5 kb and thus do not appear on the portion of the autoradiogram shown.

Table 1 Properties of small circular and linear plasmids

Plasmid	Form	Markers	% Plasmid-bearing cells	Segregation frequency	Copy no.
pSZ93	Circular	<i>ARS1</i> , <i>LEU2</i>	5	0.34	50
A75p9	Circular	<i>ARS1</i> , <i>LEU2</i> , <i>CEN3</i>	90	0.02	1
YLp4	Linear	<i>ARS1</i> , <i>LEU2</i> , <i>CEN3</i>	61	0.11	15
YLp30	Linear	<i>ARS1</i> , <i>LEU2</i>	72	0.16	50

The copy number of the plasmids was measured from Southern blots (Fig. 2) and corrected for the fraction of plasmid-bearing cells. The percentage of plasmid-bearing cells was determined by plating a selectively grown culture on nonselective medium to give about 100 colonies per plate, replica plating to medium lacking leucine and measuring the fraction of colonies which grew. The segregation frequency (see text for definition) was measured by pedigree analysis. All plasmids were present in strain A281 (*α*, *leu2*, *his3-11,15*, *can1*, *cir*^o). pSZ93 (ref. 34) is identical to A75p9 except that it lacks the *CEN3* fragment. Copy number is expressed as plasmid copy number per plasmid-bearing cell.

between 9 and 11 kb were isolated, circularized and transformed into *E. coli*. DNA was prepared from four transformants and shown to have the expected restriction map. These circular plasmids from which the Tr ends had been removed were transformed into yeast. The *Leu*⁺ transformants showed a level of mitotic stability indistinguishable from that of strains containing A75p9, proving that the *CEN3* sequences in YLp4 are still functional (data not shown). If the resolution of the Tr ends after replication in these plasmids was inhibited by the presence of a centromere, we should be able to detect unresolved replicated molecules as circular dimers on Southern blots. These were not seen even on overexposed autoradiograms. Thus, we conclude that the presence of the Tr ends inhibits the ability of *CEN3* to act as a functional centromere.

Figure 3*a* shows some of the other linear plasmids that we constructed. Their mitotic stability as measured by replica plating and by pedigree analysis is shown in Table 2. These plasmids, which range in length from 9.8 to 16.5 kb, all behave similarly to YLp4 in that they are mitotically unstable and are present in many copies.

One possibility that we considered was that the proximity of one of the Tr ends to *CEN3* in YLp4 could directly inhibit centromere function by some effect which is transmitted along the intervening DNA, such as nucleosome phasing. Centromeric DNA in yeast has been shown to have a specific chromatin structure²¹, as have the Tr ends in *Tetrahymena*²². We examined this possibility by constructing plasmids in which the separation between the centromere and the Tr ends was varied.

The smallest linear centromeric plasmid we have constructed, YLp7, has only 0.3 kb of vector DNA separating *CEN3* from

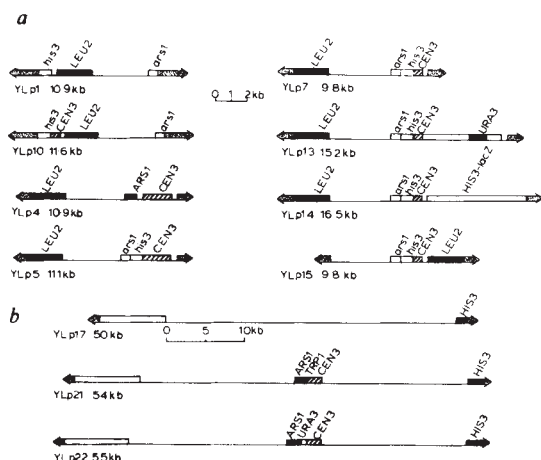


Fig. 3 Maps of linear plasmids. *a*, Short linear plasmids. The construction of YLp4 is described in Fig. 1 legend. The other plasmids were made using the same basic strategy. pBR322 sequences are shown as a single line and run through *ori* into the β -lactamase gene from left to right. Eukaryotic DNA is represented as thickened segments: —, selectable genes; —, *ARS* elements; —, Tr ends; —, *CEN3*. Non-functional genes or elements are represented in lower case letters. *b*, Long linear plasmids. Phage λ sequences are represented by a single line and run from *att* through *cos* to *J* running from left to right. Other symbols are as above.

the nearest Tr end. In YLp15 the *LEU2* fragment has been inverted so that it now lies between the centromere and the nearest Tr end. Thus, the centromere has been moved to a more central position without changing the length of the plasmid. The mitotic stabilities and copy numbers of YLp7 and YLp15 are similar (Table 2). In YLp13 a 5.2-kb fragment carrying the *URA3* gene has been inserted into YLp4 between *CEN3* and the nearest Tr end, while in YLp14 a fusion between the *E. coli lacZ* gene and the yeast *HIS3* gene (A.W.M., unpublished) has been inserted in the same position. Strains harbouring these plasmids show mitotic stabilities similar to that of YLp7 despite the greatly increased separation of the centromere from the Tr ends. In addition, *Leu*⁺ transformants carrying YLp13 are also *Ura*⁺, while those carrying YLp14 express the *HIS3-lacZ* fusion. Thus, genes on both sides of *CEN3* are expressed in these plasmids. If the Tr ends inhibit *CEN3* activity via effects which are transmitted along the DNA which lies between these elements, the inhibition is transmitted over at least 6 kb without affecting the expression of genes which lie between the Tr ends and the centromere.

Long linear centromeric plasmids

The estimated length of yeast chromosomes varies from 150 to 1,000 kb (using a value of 2.7 kb per centimorgan)²³. The plasmids described above are at least an order of magnitude shorter than the smallest yeast chromosome. We wanted to determine whether the small size of these centromeric molecules was responsible for their mitotic instability. To construct larger linear plasmids we used the strategy illustrated in Fig. 4. The λ phage λ gt4.Sc2601' (ref. 24) carries a 10.1-kb fragment of yeast DNA which contains the *HIS3* gene but lacks an *ARS* element. Phage DNA was circularized by ligation of the cohesive ends and the inverted Tr repeat was introduced into the *XhoI* site within the yeast DNA insert. As the Tr ends carry an *ARS* element, their attachment to the *HIS3* phage DNA yields unstable *His*⁺ transformants which contain linear plasmids. We named the 50-kb acentric linear plasmid YLp17 and confirmed its structure by restriction enzyme mapping on Southern blots.

The centromeric linear plasmid YLp21 was constructed by recombination *in vivo*. A strain carrying the long linear acentric

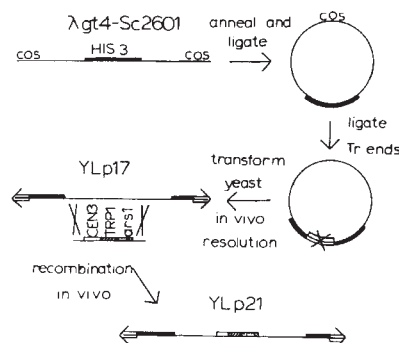


Fig. 4 Construction of a long linear plasmid. λ gt4-Sc2601' DNA²⁴ (1 μ g ml⁻¹) was annealed at 37°C for 24 h in 60 mM NaCl, 6 mM MgCl₂, 6 mM Tris-HCl pH 7.6, and then treated with T4 DNA ligase after the addition of ATP to 0.5 mM. After inactivation of the ligase, DNA was partially digested with *XhoI*, ethanol-precipitated and then ligated to the head-to-head *XhoI* dimer of the Tr ends. The ligation mix was transformed into D234.3B and *His*⁺ transformants were selected. These were screened by restriction enzyme mapping on Southern blots to identify strains carrying YLp17. TA1016, a transformant of D234.3B carrying YLp17, was transformed with a *ClaI-SphI* fragment which carries *CEN3*, *TRP1* and *ARS1* cloned into the *BglII* site at nucleotide 416 in the λ genome. This fragment was obtained by digesting the plasmid A106p1 (A.W.M., unpublished) with *ClaI* and *SphI*. *Trp*⁺, *His*⁺ transformants were selected, screened for co-instability for these markers, and finally plasmids were restriction enzyme-mapped by Southern blotting.

plasmid YLp17 was transformed by a restriction fragment which bore *TRP1*, *ARS1* and *CEN3* inserted into a segment of λ DNA. A double cross-over or gene conversion event between the restriction fragment and YLp17 serves to integrate *TRP1*, *ARS1* and *CEN3* into the *HIS3* linear plasmid²⁵. We selected transformants which were both *Trp*⁺ and *His*⁺ and screened these for ones in which the two markers co-segregated. Southern blot restriction enzyme mapping of DNA from these strains showed that they contained a 55-kb linear plasmid having the predicted restriction map. The plasmid YLp22 is similar to YLp21 but contains the *URA3* gene inserted into *TRP1*, thus inactivating it and yielding a *URA3*, *HIS3* linear plasmid of 56 kb.

The long linear centromeric plasmids YLp21 and YLp22 have mitotic segregation frequencies of 0.006 and 0.015, respectively. In selectively grown cultures, more than 95% of the cells express the plasmid-encoded markers. The long centromeric plasmids YLp21 and YLp22 are more mitotically stable than the circular *ARS*, *CEN* plasmids A106p1 and A110p1, from which their centromeres were derived (Table 2). This contrasts strikingly with the behaviour of the short linear centromeric plasmids, which are much less stable than their circular parents. To measure the copy number of YLp21 and YLp22, DNA from diploid strains which carried both of the long linear centromeric plasmids was digested with *PvuII*, run on an agarose gel and Southern-blotted using *CEN3* DNA as a probe (Fig. 5). In all eight DNA samples, representing four subclones each from the crosses DA173 and DA174, we consistently see stronger hybridization to the *CEN3* band derived from YLp21 than to that derived from chromosome III. We estimate that there are two to three copies of YLp21 per cell. In contrast, in some subclones, such as DA173S2 and DA173S4, the hybridization to the *CEN3*-containing band of the *Ura*⁺ plasmid YLp22 is of the same intensity as that to the chromosomal band, while in other subclones (DA173S1, DA173S3) the plasmid band is clearly more intense (Fig. 5). This suggests that some subclones contain two copies of YLp22 while others have only one copy, and that such differences are stable enough to persist in cultures that have been grown for at least 20 generations. These results suggest that the long linear centromeric plasmids we have constructed are faithfully

segregated to both progeny cells in most mitotic divisions. They are lost more frequently and have a less rigorously controlled copy number than do natural chromosomes.

Meiotic behaviour

When diploid yeast cells sporulate, each spore in a tetrad receives one copy of each chromosome. Sporulation of a diploid monosomic for a single chromosome produces tetrads in which two spores contain a copy of this chromosome, while the other two spores lack it and are non-viable. The behaviour of centromere-linked markers on other chromosomes demonstrates that the two viable spores are sister spores²⁶, that is, the sister chromatids of the monosomic chromosomes segregate from each other at the second meiotic division. Many monosomic strains are unstable and revert to diploidy at a high frequency²⁶. These revertants produce tetrads in which all four spores are viable.

When strains carrying circular *ARS*, *CEN* plasmids pass through meiosis, the predominant segregation classes observed are 4⁺:0⁻, 2⁺:2⁻ and 0⁺:4⁻ for the markers carried on the plasmid (Table 3)^{7,8,10,27}. 4⁺:0⁻ and 0⁺:4⁻ tetrads are presumed to arise from the sporulation of cells which contained two or no copies of the plasmid respectively, due to errors in mitotic segregation. In tetrads where the plasmid segregates 2⁺:2⁻, the two spores which receive it are sister spores, indicating that the two copies of the plasmid segregate from each other at the second meiotic division. The rare tetrads showing 3⁺:1⁻ or 1⁺:3⁻ segregation probably arise as a result of the failure of sister plasmid molecules to segregate from each other at the second meiotic division. Note that an odd number of sister or non-sister chromatid exchanges will create dicentric dimeric circles, leading to aberrant segregation.

We constructed several diploids which contained both of the long linear centromeric plasmids YLp21 and YLp22. We can regard these two molecules as being homologous artificial chromosomes because YLp22 differs from YLp21 only by the insertion of the *URA3* gene. Because this insertion lies within the *TRP1* gene, YLp21 is Trp⁺ Ura⁻, while YLp22 is Trp⁻ Ura⁺, so that the segregation of both plasmids can be followed. If the artificial chromosomes behave like natural yeast chromosomes we would expect to find two Trp⁺ Ura⁻ spores (containing YLp21) and two Trp⁻ Ura⁺ spores (containing YLp22) in each tetrad. If gene conversion between the two homologous plasmids occurred, tetrads which showed either 3:1 or 1:3 Trp⁺Ura⁻:Trp⁻Ura⁺ segregation should be found.

Table 3 shows the results of tetrad analysis of diploids containing both artificial chromosomes. For both plasmids, more than 90% of the tetrads show 4⁺:0⁻, 2⁺:2⁻ or 0⁺:4⁻ segregation. YLp21 shows a much higher fraction of 4⁺:0⁻ segregation than YLp22, consistent with its higher copy number in mitotic cells (Fig. 5a). In more than 90% of the tetrads where one or both of the artificial chromosomes segregates 2⁺:2⁻, the two spores which contain the plasmid are sisters (Table 3). This shows that the two copies of the plasmid segregate from each other at the second meiotic division, as do natural sister chromatids.

In 21% of the tetrads, both artificial chromosomes show 2⁺:2⁻ segregation. In 50 out of 55 such tetrads, the two plasmids segregate from each other, yielding two Trp⁺ Ura⁻ spores and two Trp⁻ Ura⁺ spores. Thus, the homologous artificial chromosomes segregate from each other at the first meiotic division as do natural homologues.

To investigate the correlation between the meiotic behaviour of artificial chromosomes and their copy number, we sporulated portions of the cultures used to make DNA from the subclones DA173S1 to DA173S4. The segregation of YLp21 (Trp⁺Ura⁻) and YLp22 (Trp⁻Ura⁺) in these subclones is shown in Fig. 5b. As expected for a copy number greater than one, YLp21 shows mainly 4⁺:0⁻ and 2⁺:2⁻ segregation. In DA173S2 and DA173S4, where YLp22 is present as a single copy, 2⁺:2⁻ segregation of this artificial chromosome greatly exceeds 4⁺:0⁻, whereas for DA173S1 and DA173S3, where YLp22 is present in more than one copy, there are as many 4⁺:0⁻ tetrads as

Table 2 Mitotic stabilities of linear centromeric plasmids

Plasmid	Strain	% Plasmid-bearing cells	Segregation frequency
YLp4	A281	61	0.11
YLp5	A281	56	ND
YLp7	A281	67	0.10
YLp13	A281	66	0.13
YLp14	A281	65	0.17
YLp15	A281	73	0.07
YLp21	DA173, DA174	>99	0.006
A106p1	D234.3B	91	ND
YLp22	DA173, DA174	98	0.015
A110p1	D234.3B	92	ND

A106p1 is the circular plasmid from which the centromere-containing fragment of YLp21 was obtained, while A110p1 is the plasmid from which that of YLp22 was obtained. Strain genotypes: D234.3B, α , *leu2-3,112*, *his3-11,15*, *trp1*, *ura3*, *tcm1*; DA173, see Table 3. See Table 1 legend for other details. ND, not determined.

Table 3 Meiotic segregation of circular *CEN3* and long linear *CEN3* plasmids

Diploids	Plasmid	Segregation of plasmid-borne marker % Of tetrads showing:					
		4 ⁺ :0 ⁻	3 ⁺ :1 ⁻	2 ⁺ :2 ⁻	1 ⁺ :3 ⁻	0 ⁺ :4 ⁻	% FDS*
Published data for circular <i>CEN3</i> plasmids ^{7,10}		21	7	57	1	14	98
DA163	YLp21	50	1	42	1	6	100
DA170 to DA177	YLp21	56	5	33	2	3	95
DA170 to DA177	YLp22	18	5	58	2	17	93

Data for circular *CEN3* plasmids are from refs 7 and 10. Tetrad analysis was performed as described in ref. 35. The number of tetrads analysed for the crosses shown were: *CEN3* circles, 235; DA163, 74; DA170-DA177, 259. Strain genotypes: DA163 *a/α*, *ade1*+, *leu2-3,112*+, *his3-11,15/his3-11,15*, *trp1/trp1*, *ura3*+, *tcm1*/TCM1, YLp21[*HIS3*, *TRP1*]; DA170 and DA172-DA177, *a/α*, *ade1*+, *leu2-3,112*+, *his3-11,15/his3-11,15*, *trp1/trp1*, *ura3*/ura3, *tcm1*/TCM1, YLp21[*HIS3*, *TRP1*], YLp22[*HIS3*, *URA3*]. DA171 has the same genotype as DA170 but is homozygous for trichodermin sensitivity: *TCM1*/TCM1. DA170 to DA177 were constructed by crossing Trp⁺His⁺ segregants from the cross DA163, to Ura⁺His⁺ segregants from the cross DA164, in which YLp22 was segregating. Diploids were constructed, sporulated and dissected by standard methods³⁵.

* Percentage of 2⁺:2⁻ tetrads in which artificial chromosomes are found in sister spores (first division segregation, FDS) as determined by monitoring the segregation of the centromere-linked chromosomal markers *ade1* and *leu2*.

there are 2⁺:2⁻. This suggests that the meiotic segregation of these plasmids is faithful and that the deviations from the segregation pattern expected for natural chromosomes are due to cells entering meiosis with too many or too few copies of the plasmids.

Discussion

We have constructed linear plasmids which contain all the elements known to be required for normal chromosome function. Plasmids which range from 9.8 to 16.5 kb in size are not mitotically stable and are present in many copies per cell. These plasmids behave more like acentric linear plasmids than centromeric circular ones. In contrast, linear centromeric plasmids of 55 kb constructed using phage λ DNA behave in mitosis and meiosis like circular centromeric plasmids, although they are considerably less stable than natural chromosomes. The simplest explanation of the difference in the behaviour of the two classes of linear centromeric plasmids is their difference in length. Size also affects the behaviour of natural chromosomes. The *chl* mutation leads to increased rates of mitotic loss for the smallest yeast chromosomes¹⁵. In zygotes in which nuclear fusion has been prevented by the *kar1* mutation, single chromosomes can be transferred from one nucleus to the other. The frequency of transfer decreases with increasing chromosome length²⁸.

We advance two possible explanations for the effect of length on the mitotic segregation of centromeric linear plasmids. The first is that a specific site for telomere resolution exists in the yeast nucleus, with which the ends of linear molecules remain associated even after telomere resolution. If this site is distant from the fibres of the mitotic spindle, the centromere of a plasmid or chromosome would be prevented from attaching to the spindle if the separation between the telomeres and the centromere was less than some critical distance. This would be consistent with the fact that all yeast chromosomes are metacentric as judged from their genetic maps²⁹. Although telocentric chromosomes do occur in other organisms, these have much larger chromosomes, so that the centromere may be separated from the nearest telomere by tens or hundreds of kilobases while appearing cytologically adjacent to it. Because circular centromeric plasmids are not associated with the telomere binding site, they are not inhibited from attaching to the mitotic spindle. In the interphase nuclei of polytene *Drosophila* cells, the chromocentre (where the centromeres are believed to lie) lies on the opposite side of the nucleus from the telomeres³⁰.

The second explanation invokes a requirement for the physical proximity of the two centromeres of a pair of daughter molecules to ensure their attachment to opposite poles of the spindle. For circular plasmids this proximity would be achieved if replication resulted in the production of a catenated dimer, as has been observed in the replication of simian virus 40 (SV40)³¹. In contrast, the newly replicated copies of a short linear centromeric plasmid would not be topologically constrained to remain close to each other. However, as the length of such molecules increased, the rate at which the intertwined (but not interconnected) molecules separated from each other would decline, leading to increasingly faithful mitotic segregation. This model would predict that the fidelity of segregation of linear centromeric plasmids should increase gradually with length, while the telomere attachment site model might predict that there would be a critical size range in which the mitotic stability would increase rapidly with length.

The long linear centromeric plasmids YLp21 and YLp22 behave in many respects like genuine chromosomes. However, these artificial chromosomes show a frequency of mitotic loss two orders of magnitude higher than that of natural chromosomes and a less stringent control of copy number. These differences could reflect the absence from our molecules of some previously unknown element required for proper chromosome segregation. We favour the interpretation that they indicate the suboptimal functioning of one or more of the components they contain. For example, the Tr ends may not function exactly like yeast telomeres, a larger *CEN3* fragment might be required to ensure spindle attachment at each mitosis, a second *ARS* element may be required on the opposite side of the centromere from *ARS1*, or a further increase in the physical separation of the centromere and telomeres may be needed to ensure accurate segregation.

The availability of linear molecules of defined structure whose behaviour mimics that of natural chromosomes promises to assist investigations into the mechanisms of mitotic and meiotic

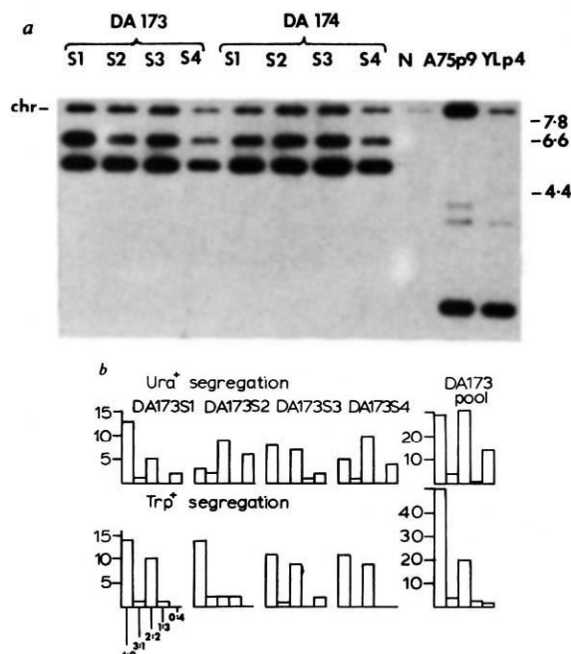


Fig. 5 *a*, Copy number of long linear plasmids. *b*, Meiotic segregation of long linear plasmids. DNA was digested with *Bam*HI plus *Pvu*II and run on a 0.5% agarose gel, Southern-blotted and then probed with a 1.2-kb *Bam*HI-*Cla*I *CEN3* nick-translated fragment. DA173 and DA174 subclones contain both YLp21 and YLp22. In these strains the lowest band is derived from the *CEN3*-containing fragment of YLp21, the middle band from that of YLp22 and the upper band from that of chromosome III. The chromosomal *CEN3* band (chr) is indicated in the left-hand margin, while the migration of molecular weight markers is shown in the right-hand margin. Other lanes: N, D234.3B without any plasmid; A75p9, strain A281 carrying the circular *ARS1*, *CEN3* plasmid A75p9; YLp4, strain A281 carrying the linear *ARS1*, *CEN3* plasmid YLp4. Faint bands in the last two lanes are due to contaminating pBR322 sequences in the radiolabelled probe. *b*, Meiotic segregation of long linear plasmids. Portions of the cultures used to make the DNAs analysed in *a* were sporulated and subjected to tetrad analysis³⁵.

chromosome segregation. By constructing artificial chromosomes which carry genetic markers near their termini, we can investigate the postulated requirement for recombination between homologous chromosomes for proper disjunction in the first meiotic division^{32,33}.

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A flare in the millimetre to IR spectrum of 3C273

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Over the past two years some of us (E.I.R., P.A.R.A., W.K.G., M.G.S., and I.G.N.) have been monitoring the millimetre and submillimetre spectrum of 3C273 (ref. 1). We have presented elsewhere a quiescent millimetre-to-far-IR spectrum² with an index $\alpha = -0.7$ ($S_\nu \propto \nu^{-\alpha}$) connecting smoothly to the radio and mid-IR. Our discovery of a concurrent flare in the IR-to-near-millimetre spectrum of 3C273 implies that the emission over this range of frequency originates in the same region of the source. The flare was seen to propagate to longer wavelengths whilst decaying at shorter wavelengths. The time scale of the flare is suggestive of an event within the central 0.1 pc of the source. Millimetre wavelength variability of 3C273 has been reported^{3,4} but with very little temporal overlap with our data. Rieke and Lebofsky¹⁶ have reviewed the 2.2 and 10 μm variability.

Between 2 and 6 February 1983 we measured the spectrum of 3C273 in 10 wavebands between 1.2 μm and 1.1 mm (J , H , K , L , M , 10, 20, 400, 800, 1,100 μm) with the United Kingdom Infrared Telescope (UKIRT) and found that its flux density at all wavelengths had increased. On 26 February, 1.3- and 2.0-mm data were obtained with the US National Radio Astronomy Observatory (NRAO) 12-m telescope at Kitt Peak. We found that the spectrum was sharply peaked at 1.1 mm, indicative of synchrotron self-absorption. During 4–7 March we again measured the 1.2 μm to 1.1 mm spectrum from UKIRT and found that there had been a further increase at all wavelengths. On 25 March we measured the 1.2–20 μm spectrum and found that the 10- and 20- μm fluxes had not changed significantly but that the J , H , K fluxes had decreased. On 9–11 April, NRAO observations showed that there had been a large increase in flux at 1.3 and 2.0 mm but a much smaller increase at 1.1 mm, suggesting that the spectrum had become optically thin at all these wavelengths. 3.3-mm observations from NRAO showed that the flux had increased by 30% from 17 March to 23 April. Six days later, UKIRT observations at J , H , K showed that the near IR had returned to the quiescent level and on 2 May the 800- and 1,100- μm fluxes were found to have decreased. Further measurements from NRAO on 20 and 22 May showed that the 1.1-, 1.3- and 2.0-mm fluxes had also decreased.

The data for all observations are presented in Table 1. For clarity of presentation, the weighted mean has been taken of all data at a given wavelength obtained during an observing run of a few days; we do not believe, within the uncertainties, that there is significant variation over this short period. The quoted uncertainties are the statistical uncertainty in an individual measurement and the uncertainty in the photometric reduction added in quadrature. Uncertainty in the absolute flux assigned to the calibrator has not been accounted for. Figure 1 shows the history of the flare using the values from Table 1. The quiescent spectrum is from the data presented in Clegg *et al.*².

The near IR (J , H , K , L) data were obtained with the common-user UKIRT photometer UKT6, with either 8 or 12 arc s apertures and chopper throws of 15 or 30 arc s at 12.5 Hz. Calibration was made with respect to the standard stars of Elias *et al.*⁵; their respective J , H , K , L flux densities were taken to be:

	J	H	K	L
HD77281	2.19,	1.48,	0.96,	0.45 Jy
HD106965	1.71,	1.14,	0.74,	0.34 Jy
BD+32954	7.01,	9.85,	7.42,	3.87 Jy

The M , 10- and 20- μm data were obtained with the common-user UKIRT photometer UKT7, with either 8 or 12 arc s apertures and chopper throws of 20 or 30 arc s at 12.5 Hz. Calibration was made with respect to the stars Alpha Boötes and Mu Ursa Majoris whose respective 10- μm flux densities were taken to be 673.3 Jy and 92.9 Jy; and at 20- μm 208.9 Jy and 30.2 Jy. The single M measurement was calibrated against Alpha Boötes whose flux density was taken to be 2,316 Jy. For none of the IR observations have corrections been applied for differences in spectral index between the calibrator and the quasar.

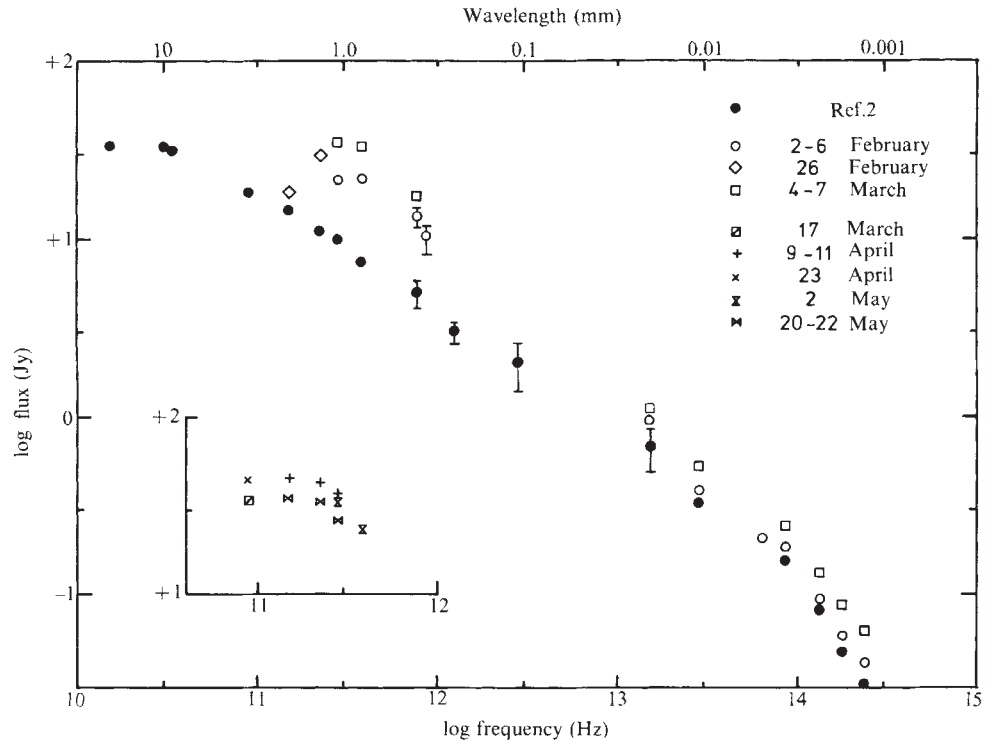
The millimetre and submillimetre data were obtained with the QMC/Oregon helium-three photometer (P.A.R.A. *et al.*, in preparation) using narrow-band filters described by Cunningham⁶ and also shown in Robson⁷. The chop throw was 120 arc s and the beam sizes were 65, 58 and 55 arc s in the 1,100-, 800- and 400- μm filter bands respectively. Calibration techniques are described in refs 8 and 9. Uranus was taken as the primary calibration standard having previously been calibrated with respect to Mars¹⁰ (M.J.G. *et al.*, in preparation), and the brightness temperatures for Uranus at the effective wavelengths of observation were: 73.9 K at 371 μm ; 84.3 K at 765 μm ; 91.1 K at 1,075 μm . Corrections for the differences in spectral slope between 3C273 and calibrators have been made for the millimetre and submillimetre data.

The NRAO observations at 2.0 mm, 1.3 mm and 1.1 mm were made with the millimetre continuum system (J.V.R. *et al.*, in preparation) mounted at the Cassegrain focus of the resurfaced 12-m dish at Kitt Peak. The average beam size was 50 arc s and the chop amplitude was 240 arc s at 6.6 Hz. Calibration was performed against Saturn and Uranus (M.J.G. *et al.*, in preparation) which had both previously been calibrated with respect to Mars and are in excellent agreement with the 3.35 mm absolute measurements of Ulich¹¹. The brightness temperatures, planetary radius and ellipticity used to deduce the flux density for Uranus were the same as used by Gear *et al.*¹. The 3.35-mm data were obtained with the NRAO 90-GHz continuum receiver with observing parameters very similar to those above. Calibration was made with respect to Jupiter.

The spectrum of the flare (after subtraction of the quiescent spectrum) can be written $S_\nu(\text{mJy}) \sim 6,000/\nu(\text{GHz})$ so that the total flux between 1 mm and 1 μm is of the order $4 \times 10^{-13} \text{ W m}^{-2}$. The total luminosity of the flare is of order $3 \times 10^{12} L_\odot$ ($H_0 = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$) giving an energy output over 2 months of around $0.1 M_\odot c^2$. There is strong evidence that the radio-to-near-IR continuum spectrum of 3C273 originates from a relativistic beam¹². In this case, the estimate for the total luminosity and energy output should be reduced by $(4\pi/\Gamma^2) \sim 0.5$, using the value 5.3 (ref. 13) for the bulk relativistic factor Γ .

Models of variability in the emission from relativistic beams due to the formation or introduction of regions of enhanced density or magnetic field in the flow have been proposed. The various predictions depend on details of the particular models and care must be taken in using our data to select a model. We believe, however, that we may exclude the shock-wave model of Blandford and Konigl¹⁴ as an explanation of the variability we have seen in 3C273. In this model, an obstacle such as a supernova remnant or an interstellar cloud enters the path of the beam, causing a hotspot to form which will emit strongly until the obstacle has been accelerated to the velocity of the

Fig. 1 History of the 3C273 flare using parameters from Table 1.



jet. Without a somewhat artificial choice of parameters (such as density of the obstacle equal to that of the jet), it is difficult to see how this acceleration can be completed in the observed time scale of about 4 months. Nor is it easy to reconcile our observations with the knot-model of Marscher¹⁵ because we do not see the steepening of the spectrum, caused by radiation losses, inherent in the model. The time scale of the flare, on the other hand, allows an upper limit of around 0.1 pc to be inferred for the linear size of the emitting region. It may not be a coincidence that this is the size expected for the central core or nozzle region of the beam. We may therefore be seeing some modifications to the dynamics or geometry at the nozzle or the

conversion of around $1 M_{\odot}$ of material by the central engine. A more detailed analysis will be presented elsewhere.

We thank the SERC for funding the observations, Mr S. Predko, Mr D. G. Vickers and Mr C. Bissick for technical assistance, Dr C. T. Cunningham for filter construction, and Dr J. Payne and Dr J. Davis for their work on the NRAO continuum receiver. We thank Drs J. Hough, W. Sparks and R. D. Joseph for allowing us to make observations during their telescope time, Mr I. Butchart for useful discussions and the UKIRT and NRAO staff for assistance. W.K.G. and M.J.G. acknowledge the support of SERC studentships. NRAO is operated by Associated Universities Inc. under contract to the NSF.

Table 1 Flux densities for 3C273

Date (UT)	mJy						Jy					
	1.25	1.65	2.2	3.45	10.5	20.0	390	790	1,100	1,300	2,000	3,350
2 February							13.3 ± 1.8	21.9 ± 0.5	20.7 ± 0.9			
3 February	39.8 ± 0.9	55.1 ± 2.3	90.1 ± 2.8	174 ± 7.6	499 ± 84	963 ± 162						
4 February					348 ± 45	942 ± 125						
5 February	43.1 ± 1.1	59.4 ± 1.2	97.6 ± 3.0	190 ± 6.6					23.2 ± 1.4			
Mean	41.1 ± 0.7	58.5 ± 1.1	93.6 ± 2.1	183 ± 5.0	382 ± 40	950 ± 99			21.6 ± 0.8			
26 February										29.6 ± 2.8	18.2 ± 0.5	
4 March							19.1 ± 4.6	37.0 ± 5.5	36.8 ± 3.0			
5 March							13.5 ± 1.9	31.2 ± 1.6	35.4 ± 0.9			
6 March	55.4 ± 3.9	78.9 ± 6.3	124 ± 10	227 ± 17			21.1 ± 2.0	32.9 ± 0.9	35.8 ± 0.9			
7 March	73.5 ± 4.4	88.4 ± 1.8	131 ± 3.9	256 ± 20	522 ± 57	1,100 ± 114	17.7 ± 1.9	32.7 ± 1.4	33.8 ± 1.9			
Mean	63.4 ± 2.9	87.7 ± 1.7	131 ± 3.6	240 ± 13			17.4 ± 1.1	32.6 ± 0.7	35.5 ± 0.6			
17 March												33.8 ± 0.4
25 March	44.0 ± 2.2	61.0 ± 3.1	96.2 ± 4.8		483 ± 48	817 ± 82						
9 April											35.6 ± 5.0	
10 April									36.6 ± 1.0	42.4 ± 0.6		
11 April											47.7 ± 2.5	
Mean											45.3 ± 2.3	
23 April												44.3 ± 1.5
29 April	35.0 ± 1.8	49.3 ± 2.5	83.3 ± 4.2	165 ± 8.2								
2 May							22.8 ± 2.1	32.9 ± 0.7				
20 May								26.0 ± 0.8		33.7 ± 1.0	35.5 ± 1.0	
22 May								26.5 ± 0.8			34.4 ± 1.0	
Mean								26.3 ± 0.6			35.0 ± 0.7	

Additional data: 3 February; 4.78 μm , 202 ± 32 mJy. 3 February; 350 μm , 9.7 ± 2.0 Jy.

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Three-dimensional simulation of large-scale structure in the Universe

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Three-dimensional numerical simulations of the nonlinear growth of adiabatic perturbations in collisionless matter demonstrate that a cellular structure develops in the Universe. This dark matter collapses into interconnecting dense regions surrounding large voids, or low density regions. The models presented here were done with a cloud-in-cell (CIC) code using a very large number, nearly 9×10^5 , of clouds. Such a large number of clouds provides adequate coverage of the voids, eliminates spurious clumping in both the high- and low-density regions, and is a significant improvement over earlier three-dimensional simulations of the cellular structure. We consider here both high ($\Omega_0 = 1.07$) and low ($\Omega_0 = 0.1$) density models. Our simulations clearly show that the interconnecting dense regions contain both filamentary and flat, pancake-shaped structures. Furthermore, the interactions among neighbouring structures are important and produce matter flows towards the dense intersections. The low-density voids which develop are approximately spherically symmetric in shape. As the voids expand, they collide and intersect. The covariance function $\xi(R)$ has a power law form at small radii, with anticorrelation found just beyond the break and no sizeable features at larger radii.

The nature and distribution of dark or 'unseen' matter in the Universe is a subject of great interest^{1,2}. Observations^{3–6} suggest that the density parameter $\Omega_0 = \rho_0/\rho_{\text{crit}}$ lies in the range $0.1 \leq \Omega_0 \leq 1$, where ρ_0 is the present total density and ρ_{crit} is the closure density. Big-bang nucleosynthesis^{7,8}, however, argues for $0.01 \leq \Omega_b \leq 0.1$, where Ω_b is the density parameter due only to baryons. Taken together, these results imply that the Universe could be dominated by dark, nonbaryonic matter.

Since this dark matter may dominate the Universe, it is important to study its dynamics. We concentrate here on the nonlinear growth of adiabatic perturbations^{9,10}, with a short wavelength cutoff λ_c , in collisionless matter. (Such a cutoff could arise, for example, by phase mixing at early times in a universe of massive neutrinos^{11,12}.) Earlier work on the adiabatic theory

in both two dimensions^{13–15} and three dimensions^{16–18} shows that a cellular structure forms in the Universe. The present two- and three-dimensional simulations and those in refs 16 and 17 were all done using the CIC technique¹⁹ on a large volume of the Universe and feature a network of many interconnected structures. The three-dimensional simulations in ref. 18 were done using direct N -body integration techniques with a much smaller number of particles. They also cover a smaller region of the Universe and show several connected structures. The present work uses an order of magnitude more particles than the largest previous study^{16,17}, allowing us to focus on the morphology of the resulting structures by clearly resolving both filaments and lower density contrast pancakes. We are able to cover the grid adequately with particles (that is, without spurious clumping) and to display pancakes clearly for the first time.

The background universe in our calculations is an expanding Friedmann–Robertson–Walker model; the Poisson equation is solved for the local gravitational potential of the dark matter. We use the CIC technique on a three-dimensional spatial grid such that there are 32 cells on a side. Our largest runs have 27 clouds per cell on average for a total of nearly 9×10^5 clouds. The CIC method was chosen to help suppress two-body scattering. This process is appropriate in modelling hierarchical clustering^{20,21} but should be suppressed to study the adiabatic theory accurately. We work in co-moving coordinates and use periodic boundary conditions. Our code runs at Lawrence Livermore National Laboratory on a CRAY-1 and was optimized (vectorized) using Stacklib software²². A run with 9×10^5 particles requires about 3 h of computer time, including disk I/O.

Our calculations start after recombination in the linear growth phase $100 < z < 1,000$. The density perturbations have very low amplitudes which mimic the Zeldovich spectrum, $|\delta_k|^2 \propto k$, with a short wavelength cutoff λ_c . We have chosen $\lambda_c = 8r_0$, where r_0 is the cell size in physical units, to ensure that there will be sufficient structures formed to model their interactions while maintaining resolution. The initial particle velocities are taken to be zero. At the start of a calculation we set $(\delta\rho/\rho)_{\lambda_c} = 0.006$ in the growing mode and choose Ω_0 ; we have run both high ($\Omega_0 = 1.07$) and low ($\Omega_0 = 0.1$) density models. This leaves r_0 and t_{start} , the time at the beginning, as two free parameters to be determined by comparing these models with observations. These two parameters take us from the dimensionless co-moving coordinates used in the calculations to dimensional astronomical quantities.

A cellular structure clearly forms in the dark matter. Figure 1 shows isodensity contours $\rho/\bar{\rho} > 2$, where $\bar{\rho}$ is the average density, at the moment when the spatial scale factor has increased by a factor of 125 for the $\Omega_0 = 1.07$ model; at this point most of the dark matter is undergoing strong nonlinear collapse $(\delta\rho/\rho)_{\text{rms}} > 1$. Note that both pancakes and filaments are present. Our large number of clouds allows us to resolve both filaments and the lower density contrast pancakes using isodensity contours; in particular, we are able to display pancakes clearly for the first time. The voids in this model at this time are shown in Fig. 2 by isodensity contours at $\rho/\bar{\rho} < 0.5$. We see from this graph that the voids are approximately spherically symmetric at this stage, leading to a 'Swiss cheese' structure. The dynamics can be described to first order by the expansion and collision of voids, with dense shells between them^{23–25}.

Another way to display the data is to plot the position of each cloud and take two-dimensional slices through the cube orthogonal to, say, the z axis. Such a slice from another of our models is shown in Fig. 3 with the arrows giving the direction of each cloud's velocity in the plane. The intersecting walls extend on neighbouring slices through about 0.25 of the cube and can be identified with pancakes. This particular model was run with only about 3×10^4 particles, that is, the same size as the largest previous study^{16,17}. Note the large evacuated regions in the voids and the spurious clumping along the bridge between the two major structures in the lower left-hand corner. These errors result from having too few particles and lead to an

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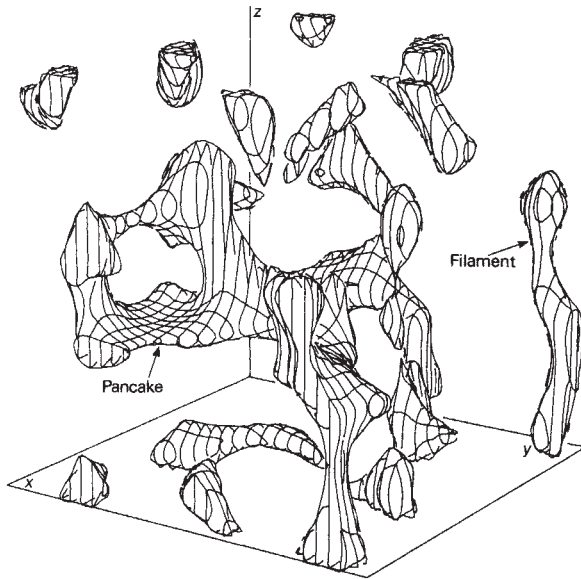


Fig. 1 A cellular structure of interconnecting dense regions forms in the dark matter, shown here on our three-dimensional spatial coordinate system. Isodensity contours $\rho/\bar{\rho} > 2$ are shown at an expansion factor of 125 for a high-density model, $\Omega_0 = 1.07$. At this stage, most of the dark matter is undergoing strong nonlinear collapse $(\delta\rho/\rho)_{\text{rms}} > 1$. Our large number of clouds, $\sim 9 \times 10^5$, makes it possible to resolve both filaments and the lower density contrast pancakes with isodensity contours.

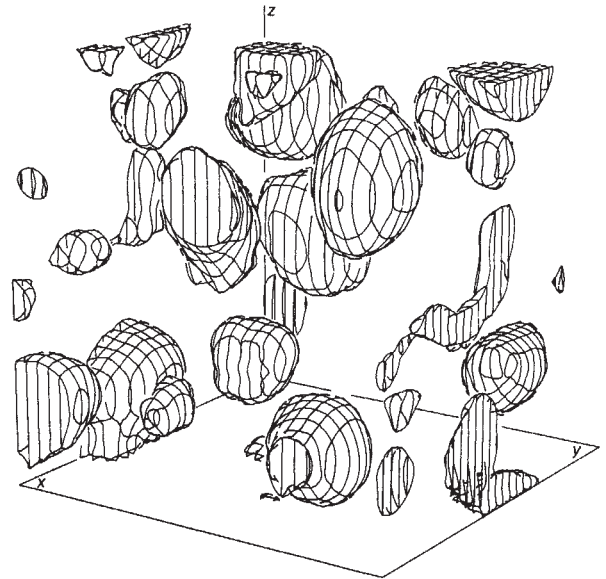


Fig. 2 The low-density regions or voids are shown on our three-dimensional spatial coordinate system at an expansion factor of 125 for the model in Fig. 1. Isodensity contours $\rho/\bar{\rho} < 0.5$ are shown. At this stage, the voids are approximately spherically symmetric. As the model evolves, the voids expand, collide and intersect.

incorrect physical model. Our larger models with $\sim 9 \times 10^5$ particles do not have these problems.

At the time of pancaking, the flat structures are at rest or expanding relative to the Hubble flow in directions transverse to the collapse axis. This is qualitatively different from the behaviour of isolated ellipsoids on a uniform background with no voids^{26,27}, and shows that objects in the cellular structure are strongly influenced by the three-dimensional structure, with both over- and under-dense regions, around them.

As the evolution proceeds, the collisionless matter flows from flat structures into filamentary ones at their intersection; thus, initially flat systems tend to evolve to thicker, more irregular ones. These matter flows leading to the dissolution of pancakes in three dimensions are analogous to those observed in earlier two-dimensional work. (See ref. 14 for a sequence of pictures demonstrating the evolution in two dimensions of planar systems to thicker ones.) Again, this process occurs because we have a system of interacting objects. The collapse of isolated pancakes does not include this important process and therefore produces systems which stay too flat²⁸. (We also find filaments which do not lie at the intersection of pancakes.)

The covariance function $\xi(R)$ at various stages in the evolution of a typical model is shown on a log-log plot in Fig. 4. At small radii, $\xi(R) \propto R^{-\gamma}$, where γ increases with evolution. There follows a break, or sudden change, in the slope on the log-log plot. Anticorrelation is found just outside this break, with no large (≥ 0.10) features in the amplitude of ξ at greater distances. For $\Omega_0 = 1.07$ we find that $\gamma \sim 1.8$ (ref. 10) at an expansion factor of about 154. At this time we find $\xi(R) \sim 1$ at $R \sim 0.2 \lambda_c$. If this is identified as the present moment on the basis of the slope of ξ , then we find $\lambda_c \sim 25 \text{ Mpc}^{10}$ and the neutrino mass $m_\nu \sim 48 \text{ eV}^{11,12}$. These values are close to those determined from a direct N -body simulation (see ref. 18). We note, however, that our value of λ_c determined in this way varies with expansion much more than in that other simulation. This discrepancy may be due to the fact that the simulation in ref. 18 uses only 1×10^3 particles while our calculations feature a larger number of structures defined by far more particles; we thereby do not 'run out' of particles during strong nonlinear collapse. All these simulations model only the dark matter, however. Preliminary calculations (in preparation) show that dissipation

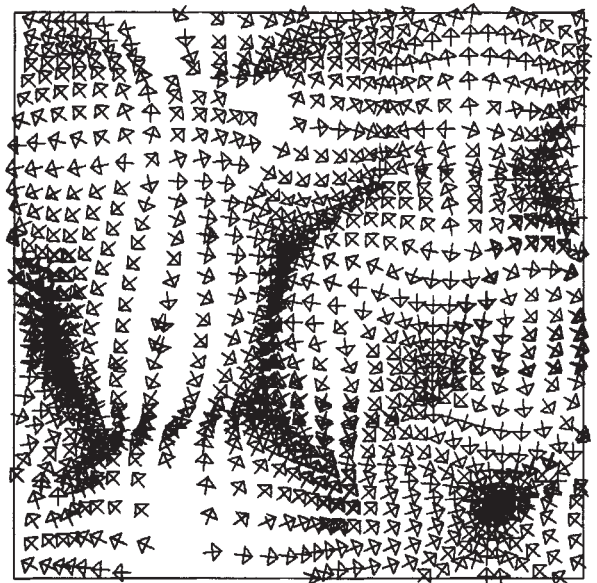


Fig. 3 A two-dimensional slice orthogonal to the z axis in the simulation. An arrow is plotted at the position of each cloud, the direction of the arrow giving the direction of the cloud's velocity in the plane. The intersecting walls on the left and in the centre extend obliquely in the z direction on neighbouring slices through about 0.25 of the cube and represent intersecting pancakes. This slice is from a model with $\Omega_0 = 1.07$ but only about 3×10^4 clouds, with all clouds in the slice being plotted. Note the evacuated regions in the voids and the spurious clumping, particularly along the bridge between the two major structures in the lower left-hand quadrant. Such clumping not only causes numerical errors but can also produce an incorrect physical model. In particular, once gas dynamical flows are added to this calculation, these clumps can act as spurious seeds for galaxy formation.

can alter both the amplitude and slope of $\xi(R)$. This effect could be important because the moment when $\gamma \sim 1.8$ in the dark matter is close to the time of pancaking (in agreement with ref. 18); however, we do not observe galaxies forming at the present epoch. Dissipation may resolve this apparent contradiction.

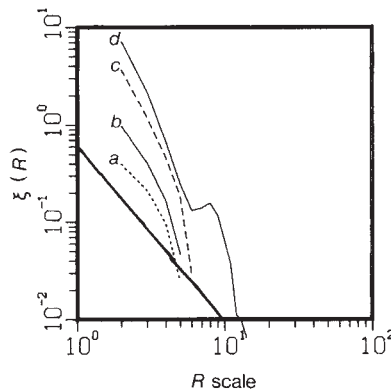


Fig. 4 The covariance function $\xi(R)$ is shown on a log-log plot for various values of the expansion factor in a $\Omega_0 = 1.07$ model. The curves correspond to expansion factors of 125(a), 200(b), 500(c) and 1,000(d). A line of slope -1.8 is shown in the lower left corner. The coordinate R gives the distance in terms of the cell size, with $R = 1$ corresponding to one cell size. Note that the curves start at $R = 2$, as this is the smallest scale that is calculated accurately.

We have also run low-density models with $\Omega_0 = 0.1$. These models also feature a cellular structure of interconnecting dense regions containing both pancakes and filaments in the dark matter. The primary difference is that the structure evolves more slowly in a low-density universe than in a high-density model, as found in the two-dimensional simulations¹³⁻¹⁵. Work is underway on a more detailed, quantitative comparison between the high- and low-density models.

In conclusion, three-dimensional simulations of the formation of large-scale structure from adiabatic perturbations in collisionless matter show that a cellular structure of pancakes and filaments forms with a power law $\xi(R)$, with a sharp break at its low-amplitude tail, and spherically symmetric voids. These features are suggestive of the observed distribution of luminous matter²⁹⁻³⁸. We point out that if this dark matter consists of neutrinos with masses $m_\nu \sim 10-100$ eV then these structures typically have masses $\sim 3 \times 10^{15} M_\odot$ and dimensions $\lambda_c \sim 10-100$ Mpc, which are characteristic of superclusters^{33,39,40}. Higher resolution calculations on a grid with 64 cells per side are in progress and models which take into account the effects of dissipative matter are planned; these will provide an even better comparison of this pure adiabatic model with observations.

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Slow neutron capture origin for $^{180}\text{Ta}^m$

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Most naturally-occurring heavy isotopes ($A \geq 74$) are believed to be synthesized by the slow (s-) and rapid (r-) neutron capture processes¹, with the rest (some 30 neutron-deficient isotopes including ^{180}Ta) being conventionally classified as p-process elements. The very rare isotope ^{180}Ta (a nuclear isomeric state $^{180}\text{Ta}^m$ with a half-life $\geq 3 \times 10^{13}$ yr)^{2,3} is of particular astrophysical interest because of its uncertain origin. Various p-process models⁴⁻⁶ (except the one eventually proposed in ref. 7) apparently fail to account even for the extremely small (2.78×10^{-6} per 10^6 Si atoms) solar abundance⁸. Another example of this kind is ^{138}La . While preserving other more specific mechanisms (such as the cosmic-ray spallation reactions^{9,10}, or the local proton irradiation in the early Solar System^{11,12}) for the possible origin(s) of these rare isotopes, we show here that a significant amount of $^{180}\text{Ta}^m$ may indeed be produced in the s-process.

Beer and Ward¹³ have examined the possibility of producing $^{180}\text{Ta}^m$ (spin-parity, 9^-)^{14,15} through a small β^- decay branching at $^{180}\text{Hf}^m(8^-)$ during the s-process and/or the post r-process cascade. A recent nuclear experimental study¹⁶ implies, however, that this scenario might explain $<50\%$ (mostly in terms of the s-process) of the solar $^{180}\text{Ta}^m$ abundance. We now consider another (and more common) type of s-process path-branching mechanism which may be responsible for the formation of $^{180}\text{Ta}^m$.

Figure 1 displays *a*, the proposed s-process branching path and *b*, the reaction chain of main interest here: β^- decays of the ^{179}Hf excited states thermally populated at s-process temperatures (a few times 10^8 K), followed by neutron captures on the daughter ^{179}Ta , leading partly to $^{180}\text{Ta}^m$. We treat $^{180}\text{Ta}^m$ as a separate isotope as no direct or indirect transitions^{17,18} in

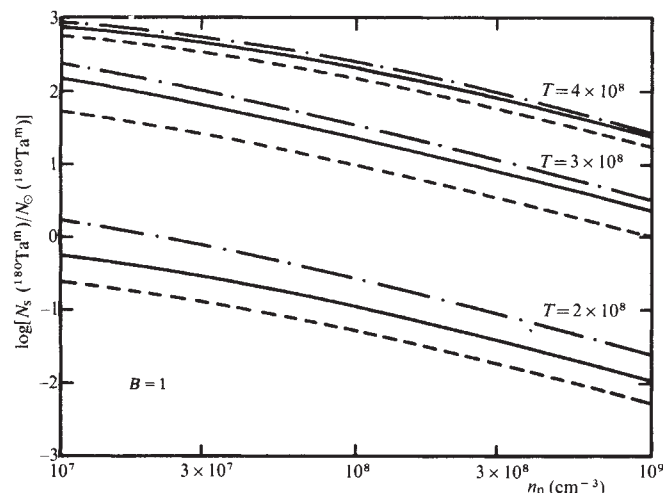


Fig. 3 The amount of $^{180}\text{Ta}^m$ synthesized by the s-process branching of Fig. 1 [relative to the solar value $N_{\odot}(^{180}\text{Ta}^m)$], displayed in the case $B = 1$ as a function of the neutron number density n_n for given temperatures T (K) and mass density ρ (g cm^{-3}) of 10^3 (dot-dashed-curve), 3×10^3 (solid curve) and 10^4 (dashed curve).

quantum numbers¹⁵. (In the case of $^{181}\text{Ta}(n, \gamma)$, the same method gives only a fractional population of 5×10^{-4} and 2×10^{-20} to $^{182}\text{Ta}^{m2}(10^{-}, \sim 520 \text{ keV})$ through the 3^+ and 4^+ compound state, respectively, which is comparable to the thermal neutron value²² of $5 \times 10^{-20}\%$.) While a more detailed calculation which also takes into account the first excited ($\sim 31 \text{ keV}$) state of ^{179}Ta is called for, the present results make it plausible that a large part of the solar ^{180}Ta abundance can be produced in the s-process branching considered here.

Our model also leads to an s-process contribution

$$N_s(^{180}\text{W}) = (1 - B)f_{-}(^{180}\text{Ta}^g) \times [\bar{\sigma}_{30}(^{180}\text{Ta}^m)/\bar{\sigma}_{30}(^{180}\text{W})]N_s(^{180}\text{Ta}^m; B = 1) \quad (2)$$

to ^{180}W , which is usually classified as a p-process element. In equation (2), $f_{-}(^{180}\text{Ta}^g)$ is the β^- decay branching ratio of the ground state (plus its companion states) of ^{180}Ta , and $N_s(^{180}\text{Ta}^m; B = 1)$ is the upper limit of the amount of the synthesized $^{180}\text{Ta}^m$ (Fig. 3). This $N_s(^{180}\text{W})$ should not exceed the solar value $N_{\odot}(^{180}\text{W}) = 1.78 \times 10^{-4}$ (ref. 8), and probably should not be so large as to disturb p-process systematics. In return, these limits will eventually constrain s-process conditions.

Figure 4 displays $N_s(^{180}\text{W})/N_{\odot}(^{180}\text{W})$ calculated with those sets of T and n_n for which $N_s(^{180}\text{Ta}^m)$ is solar, while ρ is taken to be $3 \times 10^3 \text{ g cm}^{-3}$. [The post s-process $^{180}\text{Ta}^g\beta^-$ decay contributes insignificantly to $N_{\odot}(^{180}\text{W})$.] As for the unknown cross-section $\bar{\sigma}_{30}(^{180}\text{W})$, we have adopted 500 mb as a representative value roughly evaluated from the systematics, while a theoretical calculation²⁸ predicts 1,090 mb. (Note that $N_s(^{180}\text{W})$ is inversely proportional to $\bar{\sigma}_{30}(^{180}\text{W})$.)

For a given measurable quantity B , only the right-hand side (high n_n) of the corresponding curve in Fig. 4 is allowed to be the T - n_n domain for the s-process, lest the calculated $N_s(^{180}\text{Ta}^m)$ exceed the solar value $N_{\odot}(^{180}\text{Ta}^m)$. Should the present scenario explain all the $N_{\odot}(^{180}\text{Ta}^m)$, a stringent relation between T and n_n in the s-process would be obtained as shown. In considering that ^{180}W seems to be easily produced in the p-process (see ref. 6), let us assume that the s-process contribution $N_s(^{180}\text{W})$ should not exceed, say, a quarter of the solar value $N_{\odot}(^{180}\text{W})$. This constraint seems to be reasonable in view of the remaining uncertainties in the p-process models. If so, the $B = 0.1$ curve is in the allowed domain, but not the $B = 0.05$ curve. In the latter case, a compromise may be found in the higher n_n region where, for the same B , $N_s(^{180}\text{Ta}^m)/N_{\odot}(^{180}\text{Ta}^m)$

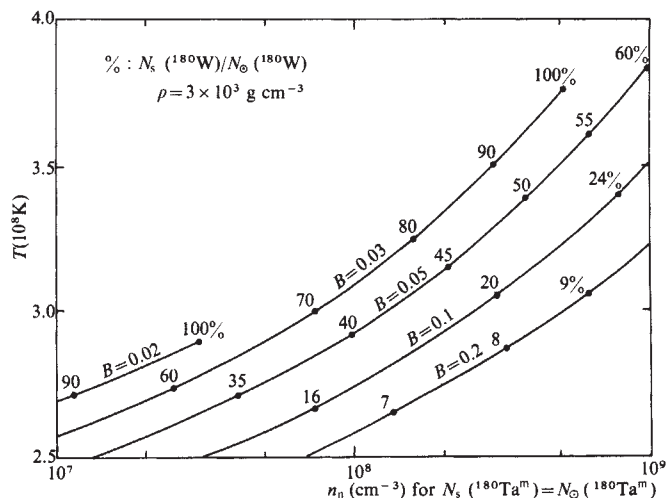


Fig. 4 The amount of ^{180}W [normalized to the solar value $N_{\odot}(^{180}\text{W})$] produced in the s-process along with $^{180}\text{Ta}^m$. The curve for a given B provides the relation between T and n_n values for which $N_s(^{180}\text{Ta}^m) = N_{\odot}(^{180}\text{Ta}^m)$. The mass density ρ is fixed at $3 \times 10^3 \text{ g cm}^{-3}$. The calculated $f_{-}(^{180}\text{Ta}^g)$ are considerably larger than the terrestrial one quoted in Fig. 1b because the net effect of high ionization in the s-process conditions is to hinder the electron captures (neutron captures hardly competing with β transitions). These results are obtained with $\bar{\sigma}_{30}(^{180}\text{W}) = 500 \text{ mb}$ (see text).

is smaller than unity and thus $N_s(^{180}\text{W})/N_{\odot}(^{180}\text{W})$ is decreased (see, equation (2)).

We have shown that the $^{179}\text{Hf}(\beta^-)^{179}\text{Ta}(n, \gamma)^{180}\text{Ta}^m$ branching in the s-process might significantly contribute to the solar ^{180}Ta abundance. To confirm this conclusion, a clarification of the astrophysical site(s) for the s-process, as well as improved nuclear data, are required. The astrophysical sites for the production of the solar s-process abundances are still under debate³⁰. We have adopted here the steady flow model with constant T and n_n (which might eventually mimic various astrophysical environments for the s-process: see ref. 31 for a discussion), whereas the most extensively studied s-process model deals with the pulsed neutron irradiation accompanying the recurrent He shell thermal pulses in intermediate stars^{32,33}. In this latter model, T and n_n are expected to decrease dramatically during the course of each pulse. In such a situation, the present results cannot be directly applied.

From a nuclear physics viewpoint, cross-section measurements of the $^{179}\text{Ta}[1.7 \text{ yr}](n, \gamma)^{180}\text{Ta}^g$ (which determine the key quantity B), $^{180}\text{Ta}^m(n, \gamma)^{181}\text{Ta}$ and $^{180}\text{W}(n, \gamma)^{181}\text{W}$ reactions at the astrophysical energies are encouraged. In particular, we have shown that an experimental determination of the B value (or at least its upper limit) would help constrain acceptable s-process conditions. A mapping of such constraints based on s-process path-branchings at different isotopes (see refs 31, 34, 35) is to be initiated. Finally, we note that a similar branching in the s-process cannot account for the solar ^{138}La abundance to any significant level.

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Thermally-activated creep and flexure of the oceanic lithosphere

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Thermal models of the oceanic lithosphere^{1–4} explain ocean floor bathymetry, heat flux and gravity and geoid⁵ anomalies by the cooling and thickening of the lithosphere as it ages away from ocean ridges. Surface wave studies^{6,7} tend to confirm that thickness increases with thermal age. Thickness estimates based on the lithosphere's flexural response, parameterized in terms of an elastic model floating on a inviscid substratum, are, however, factors of two to three times less than those from the thermal and seismic models^{8,9}. We show here that a unification of estimates can be achieved by a different, yet simple, parameterization of the bending response of the lithosphere. The lithosphere is treated as a non-convective layer that undergoes thermally-activated creep according to its temperature at the time of loading. The solutions to this problem for loads emplaced on lithosphere of increasing age are then used as a basis set to construct the solution to the problem of stress relaxation in a thermally ageing lithosphere. We show that the predicted deformation passes through a series of apparently weaker quasi-elastic states, which can be equated with those of the elastic thin-plate flexural models, but the process is controlled by the thermal lithosphere and its associated thickness. The controlling parameters are the basal viscosity of the lithosphere and the activation energy for creep in mantle rocks.

Temperature increases rapidly with depth across the lithosphere to $\sim 1,350 \pm 100^\circ\text{C}$ at depths ranging from as little as 10 km beneath ocean ridges to ~ 200 km beneath the oldest shield regions. Laboratory experiments and theory have shown that creep is a thermally activated process for mineralogies thought to be representative of this layer. The creep law takes an exponential form in temperature¹⁰,

$$\dot{\epsilon} = f(\sigma, d, T) \exp[-(E^* + PV^*)/RT] \quad (1)$$

where $\dot{\epsilon}$ is the deviatoric strain rate; σ is the deviatoric stress tensor; d , the grain size; T , the absolute temperature; E^* , the activation energy, and V^* , the atomic volume for diffusion of

the rate limiting species; P , the ambient pressure; and R , the gas constant. No matter whether $f(\sigma, d, T)$ is linear, as we assume, or power law in stress, the effective viscosity, $\eta = \sigma/\dot{\epsilon}$, within the lithosphere will decrease by more than 10 orders of magnitude from top to bottom purely as a result of the temperature dependence. The implication is that creep rates induced by loading of oceanic islands will vary rapidly with depth such that the lower lithosphere will relax stress on a time scale of $\sim 10^5$ yr, similar to that of glacial rebound which samples mantle viscosities, whereas near surface regions are too cold to allow stress relaxation by viscous flow on time scales as long as the age of the Earth, $\sim 10^{10}$ yr (ref. 11).

Models of flexure which represent the lithosphere as having constant properties with depth are an oversimplification. Elastic models¹² correctly represent only the rigid near-surface regions and therefore characterize the asymptotic properties, whereas uniform viscoelastic plates^{13,14} cannot correctly represent relaxation in terms of a constant viscosity with depth distribution. The poor resolution of flexural studies of the depth variation in viscosity makes any attempt at inversion of multilayer models for specific parameter values associated with each layer unrealistic. Multilayer, multiparameter models are therefore of limited use. We propose a viscoelastic Maxwell model which, although solved numerically through a multilayer approach, is conceptually simple and has only two parameters, the basal viscosity, η_b , and the activation energy for creep, $Q = E^* + PV^*$. Other properties are taken directly from the thermal models of the lithosphere. This approach may be used in flexural studies of oceanic lithosphere and the subsidence of sedimentary basins (our work, in preparation). The Maxwell rheology allows creep by viscous flow of stresses that are propagated elastically. It therefore corresponds to the Earth much more closely than, and has different response characteristics from, a purely viscous model¹⁵.

The mathematical development of the time-independent viscoelastic model¹⁵ closely follows that of Peltier¹⁶ and Wu and Peltier¹⁷ except that a non-gravitating layered half-space is used instead of a self-gravitating sphere. The steps are as follows. The viscoelastic problem is transformed through the correspondence principle¹⁶ and the Laplace transform to the equivalent elastic problem which is independent of time but dependent on the Laplace transform variable. The elastic-like problem, deformation of a multilayer overlying a half-space, is then solved for a range of axisymmetric Bessel function surface loads by propagating the matrix form of the solution from the basal half-space through the welded layers to the surface. This is done for a complete spectrum of the Laplace transform variable and range of the Hankel wavenumber. The result at this stage is the doubly integral transformed Laplace and Hankel form of the solution in time and space. The Laplace transform for each Hankel wavenumber may be inverted through a normal mode formalism¹⁷ in which the eigenvalues are poles of the integrand which defines the inverse Laplace transform. The poles lie on the negative real axis in the complex plane. The Laurent series therefore takes on decaying exponential form in time with time constants given by the pole positions and amplitudes given by the values of the residues at the poles (Fig. 2). The inverse Hankel transform completes the solution in which the load is assumed to take a step function form in time. A weighted sum of the solutions for each wavenumber is used to create a delta-function load in space, if the space-time Green functions are needed, or a conic of gaussian cross-section may be used to represent a seamount.

The elastic rigidity is assumed to be 2.7×10^{10} Pa throughout the model, but the viscosity, η , varies with depth, z , and time, t , according to a simple parameterization of equation (1),

$$\eta(z, t) = \eta_b \exp\left(\frac{Q}{R}\left(\frac{1}{T(z, t)} - \frac{1}{T_m}\right)\right)$$

where $T(z, t)$ is the temperature, R the gas constant, and T_m and η_b are the temperature and viscosity of the half-space below the lithosphere. This variation is shown for bounding values of

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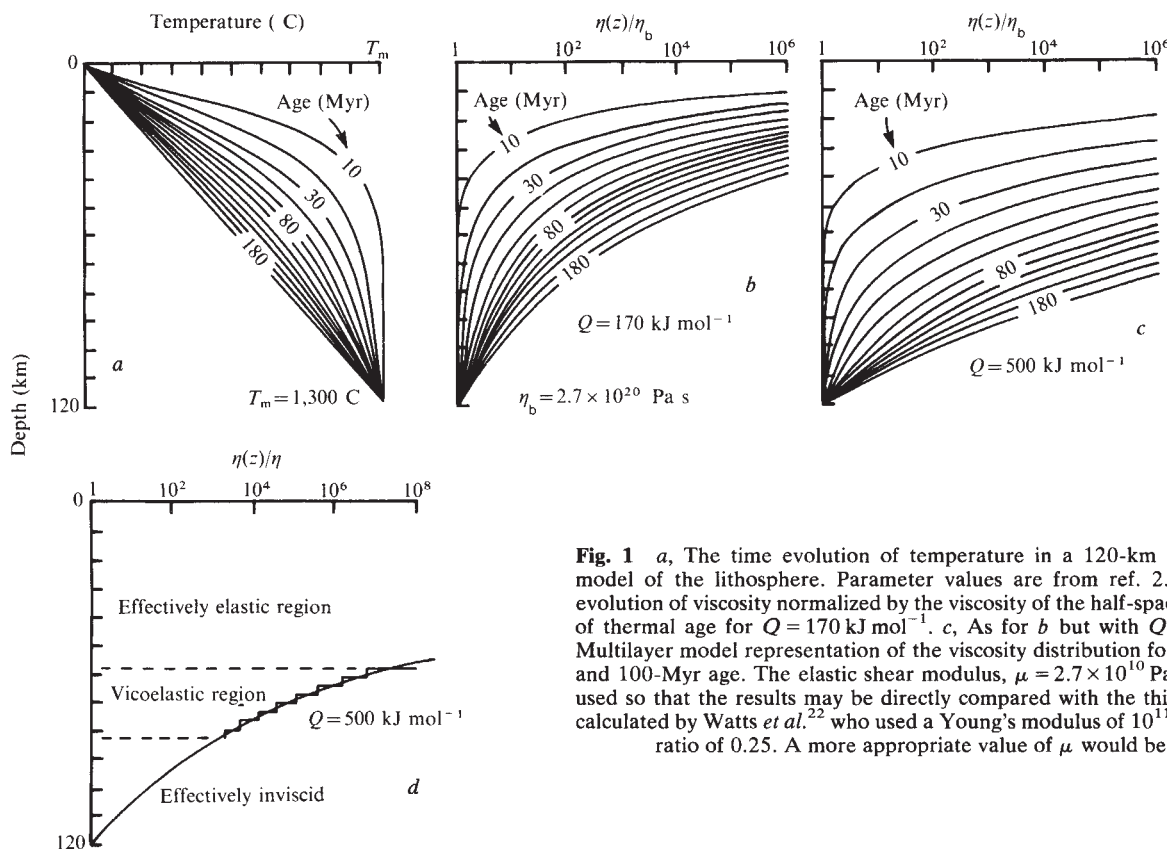


Fig. 1 *a*, The time evolution of temperature in a 120-km thick cooling plate model of the lithosphere. Parameter values are from ref. 2. *b*, Corresponding evolution of viscosity normalized by the viscosity of the half-space, η_b , as a function of thermal age for $Q = 170 \text{ kJ mol}^{-1}$. *c*, As for *b* but with $Q = 500 \text{ kJ mol}^{-1}$. *d*, Multilayer model representation of the viscosity distribution for $Q = 500 \text{ kJ mol}^{-1}$ and 100-Myr age. The elastic shear modulus, $\mu = 2.7 \times 10^{10} \text{ Pa}$. This low value is used so that the results may be directly compared with the thin plate thicknesses calculated by Watts *et al.*²² who used a Young's modulus of 10^{11} Pa and a Poisson's ratio of 0.25. A more appropriate value of μ would be $\sim 10^{11} \text{ Pa}$.

Q , $\eta_b = 2.7 \times 10^{20} \text{ Pa s}$ (Fig. 1*b, c*) and temperatures (Fig. 1*a*) predicted by Parsons and Sclater². The model (Fig. 1*d*) assumes: that effectively infinite viscosity regions can be represented by an elastic layer; each of the uniform layers has a uniform viscosity equal to the logarithmic mean of the continuous distribution; and that rapidly relaxing regions that leave no geological record may be included in the half space.

There are three relaxation pole series for a typical time-independent viscosity model (Fig. 2): the L , or layer, poles, of which only one of the two for each layer is important; the M_0 poles, arising from the basal half-space; and the M_1 poles that appear because there is an elastic lid. The M_0 series contributes most to the residues because most of the viscous flow is in the half-space but the L and M_1 series give peak contributions at wavenumbers, $k \sim 1/d$, where d is the effective thickness of the layer. The amplitude of each residue determines the extent to which the associated relaxation mode will appear in the geological record. For example, the high wavenumber decay of residues reflects the increasing flexural support by the elastic region of the lithosphere with increasing wavenumber.

The surface deformation in the wavenumber domain has been calculated as a function of time for models with a fixed η_b , but variable Q , and time-independent $T(z)$. A suite of thermally ageing $T(z)$ models therefore represents how the lithospheric response would change were loads placed on a lithosphere that simultaneously stopped ageing. A least-squares fit in the wavenumber domain has been used to estimate the equivalent thickness of a thin elastic plate as a function of time after the load is applied. The progressive 'up from the bottom' style of relaxation means that the response spectrum is closely approximated by a progressively-thinning elastic plate and that the equivalent elastic thickness is a good way to characterize the results (Fig. 3). Thicknesses decrease, rapidly at first and then more slowly. The thickness of the region that relaxes stress increases with increasing thermal age of the lithosphere as does the thickness of the effectively elastic region.

The 180-Myr old model closely represents the predicted response of thermally mature oceanic lithosphere and it can be seen that the equivalent thickness is always much less than the

125 km of the mature thermal lithosphere. $Q \sim 170\text{--}250 \text{ kJ mol}^{-1}$ predicts the observed degree of effective thinning.

A suite of these models can be used as a basis set to approximate in a stepwise fashion the relaxation of a lithosphere that

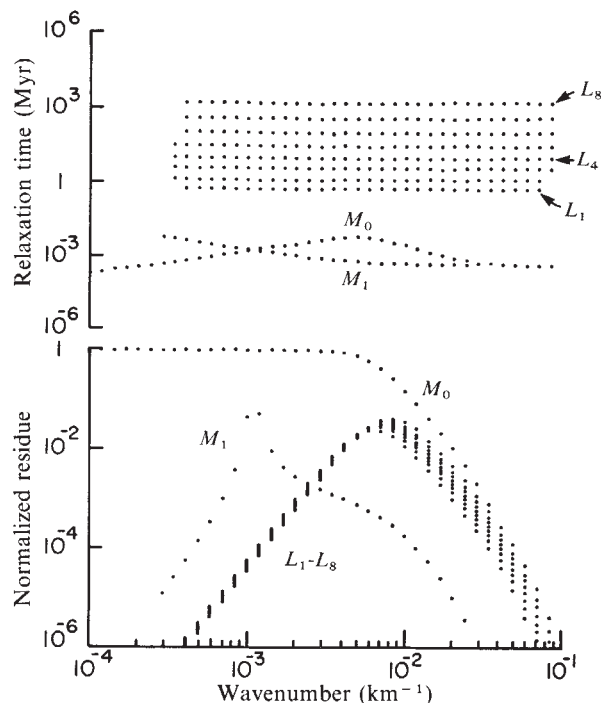


Fig. 2 The poles and residues for an eight-layer viscoelastic model plotted as a function of wavenumber. $Q = 500 \text{ kJ mol}^{-1}$, $\eta_b = 10^{21} \text{ Pa s}$, mantle density $= 3,400 \text{ kg m}^{-3}$, and the thermal age of lithosphere $= 100 \text{ Myr}$. The M -modes are from the underlying half-space relaxation. The L_1 layer is the layer immediately above the half-space. The Heavyside residues are normalized by the difference between the local isostatic deformation and the initial elastic deformation¹⁶.

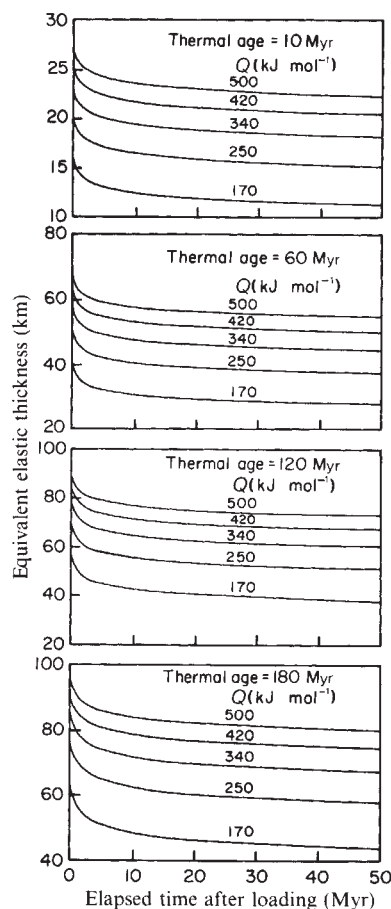


Fig. 3 The decrease in equivalent elastic thin plate thickness as a function of elapsed time since loading for models of differing initial thermal age and Q varying from 170 to 500 kJ mol^{-1} . $\mu = 2.7 \times 10^{10} \text{ Pa}$; $\eta_b = 2.7 \times 10^{20} \text{ Pa s}$; mantle density = $3,400 \text{ kg m}^{-3}$. The equivalent thickness is a measure of the way in which the lithosphere apparently thins following loading.

continues to mature after loading. The method relies on conserving potential energy between progressively older models at intervals reflected by the finite age steps in the suite of models¹⁵. For example, the load is allowed to relax on a 10-Myr model for 5 Myr after which its uncompensated part is transferred to a 20-Myr old model for the next 10 Myr and so on. This approach is strictly valid for a viscous material but can also be shown to be accurate for the Maxwell rheology when viscosity increases rapidly towards the surface, as it does in these models. The best-fitting equivalent elastic plate thickness was found in the same way for each of the composite relaxation spectra as a function of age of the plate and elapsed time since the plate was loaded. The large elapsed time asymptote of these results for a range of Q and η_b values (Fig. 4) shows the ultimate equivalent elastic thickness versus thermal age of the lithosphere when loaded. Little relaxation occurs after 5 Myr of loading. The thickness increases with age for all models showing the dominant effect of cooling in reducing viscous flow; the stresses are frozen into the lithosphere. The dashed lines show the predicted elastic thickness if the Earth is regarded as truly elastic to the 300 or 600 °C isotherms and inviscid below that. These are the bounds that best agree with data fitted to elastic thin plate models of flexure of the oceanic lithosphere⁸.

Bounds on acceptable Q , η_b , pairs are shown in Fig. 5. These are modified if the uppermost regions of the lithosphere are fractured and cannot sustain stress. As an example, we give the equivalent results when such a 'rubble' layer 10 km deep exists. This thickness represents an upper bound chosen to correspond

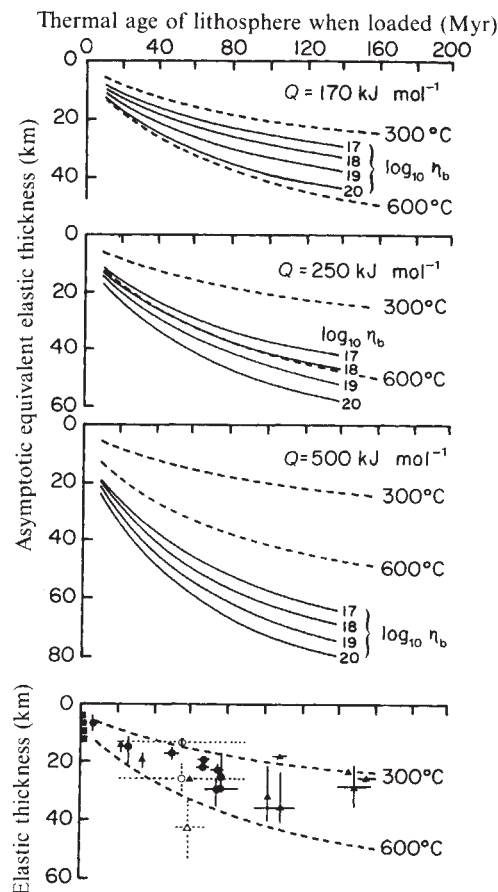


Fig. 4 The asymptotic equivalent elastic thin plate thicknesses that are frozen into the cooling lithosphere plotted against thermal age of the lithosphere when loaded for a range of values of Q and η_b . The 300 and 600 °C isotherm positions for lithosphere as a function of thermal age are also shown. These isotherms bracket the thin elastic plate estimates of lithospheric thickness for oceanic lithosphere from Bodine *et al.*⁸ (shown in the bottom panel) and therefore the region in which successful models must plot.

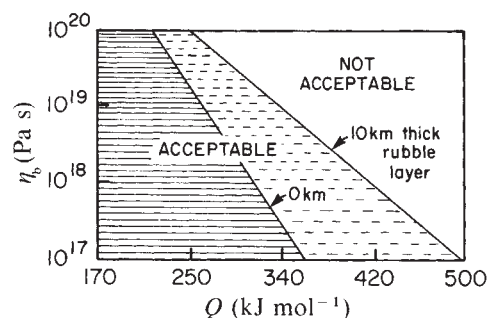


Fig. 5 Bounds on acceptable model parameters Q and η_b from a comparison with observations⁸. Note the increase in acceptable values when the upper 10 km of the lithosphere are considered to be a pervasively faulted 'rubble' layer that is incapable of sustaining deviatoric stress. This choice represents an upper bound on the properties and thickness of such a layer.

to the region in which seismic activity occurs in oceanic crust away from subduction zones.

The implication of the results is that the proposed simple model provides a unification of thermal and mechanical views of the lithosphere. The mechanical properties of the lithosphere follow naturally from its thermal properties as a consequence of the thermal activation of viscous flow. Only two continuum parameters, Q and η_b , are needed to characterize these mechanical properties and there is no dichotomy between the

thermal and flexural thickness. We believe that this improved parameterization has significant advantages over the elastic and uniform viscoelastic plates yet remains sufficiently simple in construction and number of parameters to be of use in tectonic models involving lithospheric flexure.

The universality of values for Q and η_b provides the major test of the model. That the preferred values of $Q \sim 170$ – 250 kJ mol^{-1} are $\approx 350 \pm 50 \text{ kJ mol}^{-1}$ (refs 18–20) observed in the laboratory for creep controlled by 'wet' olivine has also been found^{20,21} and may be due to nonlinear creep; reflect the difference between polycrystalline and single crystal creep; be caused by impurities; or be a consequence of significant reheating, during the intrusion of seamounts, which resets the thermal age of the lithosphere. Furthermore, the results cannot be attributed to flow within the crust, for which low values of Q would be more appropriate, because the oceanic crust chills rapidly to temperatures at which no viscous creep occurs. It is also unlikely that significant brittle fracturing, which would reduce effective thickness, occurs outboard of seamount loads in contrast to subduction zones²¹.

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Possible heterogeneity of the Earth's core deduced from PKIKP travel times

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The core of the Earth is usually described by spherically-symmetrical velocity models. The core is made of two main spherical layers: the fluid outer core with a radius close to 3,480 km and a P-velocity increasing with depth from 8 km s^{-1} to 10.3 km s^{-1} , and the solid inner core with a radius of 1,220 km and a P-velocity close to 11 km s^{-1} (refs 1, 2). Station residuals of the seismic core phase PKIKP have been computed for 400 seismological observatories worldwide using 5 yr of the International Seismological Centre (ISC) Bulletins. PKIKP travel times can be corrected for upper mantle propagation by subtracting P delays; thus PKIKP–P residuals are a measurement of the average vertical travel times in the lower mantle and in the core of the Earth beneath seismic stations. A spherical harmonic development of PKIKP–P delays up to degree 4 explains 58% of the variance in the data. PKIKP–P exhibit a latitudinal dependence: polar stations tend to be faster than equatorial stations. We show here that this pattern may reflect a departure from spherical symmetry in the P-velocity distribution in the vicinity of the inner core boundary of the Earth.

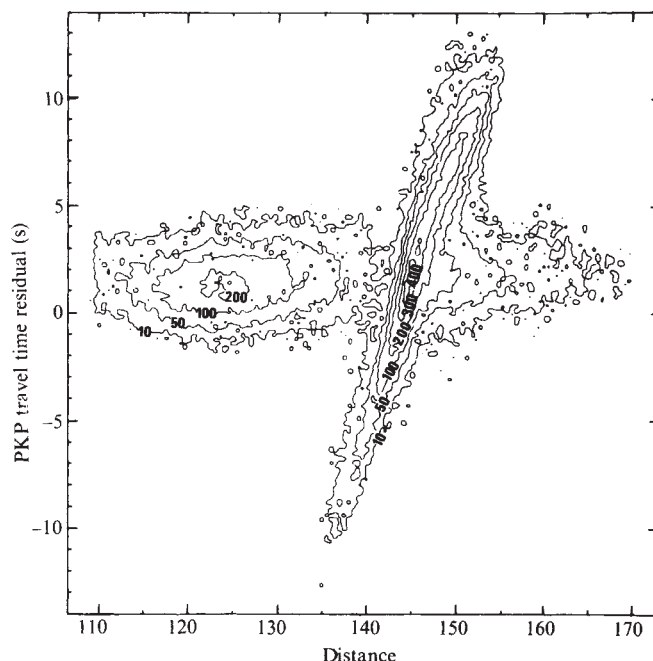


Fig. 1 Travel-time residuals in seconds of the core phase reported as PKIKP by the ISC, as a function of epicentral distance. The PKIKP residuals are computed with respect to Jeffreys–Bullen tables.

The ISC collects readings of P arrival times for all major earthquakes and locates them with Jeffreys–Bullen's tables³. Core phases are reported but not used in the computation of the hypocentres; among them, PKIKP is a wave entering the inner core. PKIKP delays are computed with respect to Jeffreys–Bullen's tables for core phases using Shimshoni's⁴ depth corrections. PKIKP or PKP (DF) travel times have been extracted from 5 yr of ISC tapes⁵ and Fig. 1 shows all phases reported as PKIKP. The coordinates are the difference in arrival time between the observed PKIKP and the theoretical travel times, and the epicentral distance in degrees. The number of PKIKP arrival times in cells of 0.2 s and 0.5° is plotted with level lines at 10, 50, 100, 200, 300 and 400. Four hundred observations are reported at 143° for a PKIKP delay of 1.5 s. The PKIKP diagram should be a horizontal linear ridge with a mean value of 1.5 s. The main feature observed on Fig. 1, beside the expected horizontal DF ridge, is a narrow ridge transversal to the DF branch. Short period precursors to PKIKP in the distance range 120–143° have been interpreted as: (1) one or several refracted branches due to one or several small velocity gradients in the outer core^{6–8}; (2) energy diffused by heterogeneities in the lower mantle near the core–mantle boundary⁹; this hypothesis clearly explains the small and weak precursors which are reported at distances <135° and which are spread in time; (3) diffraction from the PKP caustic B^{10,11}. Hypotheses (1) and (2) have already been invoked to interpret the transverse ridge from Fig. 1 obtained from limited sets of data^{12,13}. The transverse ridge is precisely timed and a ray parameter of 3.4 s per deg is observed. On long period data, the diffraction from PKP at B has a ray parameter of 3.43 s per deg (ref. 14): therefore the most probable interpretation of the transverse branch in the PKIKP reports of the ISC is that it is the short period diffraction from PKP caustic B. Such an interpretation does not exclude the existence of phases related to hypotheses (1) and (2): the ISC only reports first arrivals and, on seismograms, energy is often observed in the time interval between PKIKP and the transverse branch of Fig. 1.

The multibranching in Fig. 1 makes the computation of PKIKP station residuals more complex than the computation of P station residuals. Usually a P or PKIKP residual is an arithmetic mean of travel-time residuals computed for various

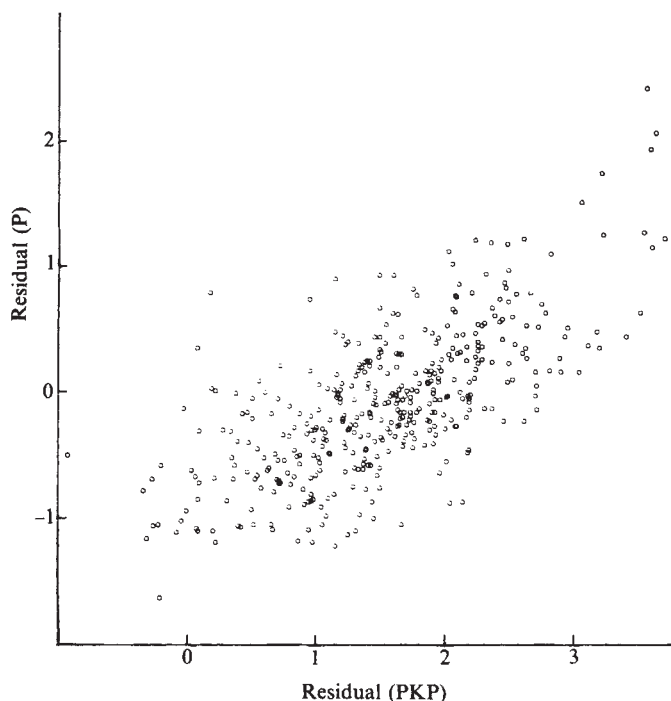


Fig. 2 Correlation between P station residuals and PKIKP station residuals for seismological observatories worldwide. Both axes are graduated in seconds.

earthquakes¹⁵. Weighting and special tests are applied to eliminate dubious data. To compute PKIKP station residuals from the data presented in Fig. 1, we should eliminate the transverse branch. The first solution would be to eliminate data from 135° to 155°, but the number of data would be too small. To get rid of arrivals that do not belong to the DF branch, we do not retain data from between two parallel lines: $R = 1.6 \times \text{distance} - 223.165$ and $R = 1.6 \times \text{distance} - 240.2$, where R is in seconds and the distance is in degrees. A histogram of residuals is then computed for each station and data outside the ± 3 s interval around the median are rejected. A PKIKP station residual is computed as the arithmetic mean of the remaining data. Figure 2 shows PKIKP station residuals plotted as a function of P residuals⁹: the mean value for PKIKP is 1.5 s

and P and PKIKP are correlated within a band of 2 s. A P station delay is essentially a measurement of the vertical P travel time in the upper mantle beneath the station, whereas PKIKP always remains in a narrow cone beneath the station and is sampling the average structure of the upper mantle as well as deeper parts of the Earth.

To correct PKIKP times for propagation in the upper mantle, we compute PKIKP station residual minus P station residual: in theory these delays should give an average propagation time in the lower mantle and in the outer core. Figure 3 is a histogram of PKIKP-P residuals for 400 seismological observations worldwide. The cells are 0.1 s wide. Most PKIKP-P are within an interval of 0–3 s. The seismic stations are referenced by their ISC codes. The errors on PKIKP are of the order of 0.5 s for a station that recorded 100 arrival times and many stations reported more than 100 PKIKP⁵.

PKIKP-P are geographically correlated. Late region (larger number) are found in South America, for stations which are not in the same tectonic environment. South American stations report a large number of core phases and their average PKIKP-P is well defined. The trend in the data is to indicate slower propagation times near the Equator compared with faster travel times near the North and South Poles.

The PKIKP field was filtered by performing a spherical harmonics expansion up to a degree 4 (Fig. 4). A simplified pattern very similar to the one observed from the raw data explains 58% of the variance in the data. It exhibits a strong latitudinal dependence: two slow regions are observed near the Equator in South America and near the coast of Brazil for the first region and near the Philippines for the second region. The fastest regions are near the Bering Sea, Alaska and Northern Canada and centred between New Zealand and Antarctica. The travel time difference between slow and fast regions is of the order of 2 s and is resolved by the data. Central Asia and Europe do not show any clear trend.

The first guess, considering such a pattern, is that PKIKP data were not corrected for ellipticity: large travel times would be observed at the Equator and small ones near the poles. But the ISC applies Bullen's¹⁶ ellipticity corrections to all phases reported in the bulletins and therefore there should not be any major latitudinal dependence of the PKIKP residuals. The existence of a systematic bias in PKIKP due, for instance, to a poor distribution of sources and receivers, cannot be disproved. However, results on P residuals, processed in the same manner, show that travel-time delays computed from raw ISC data are

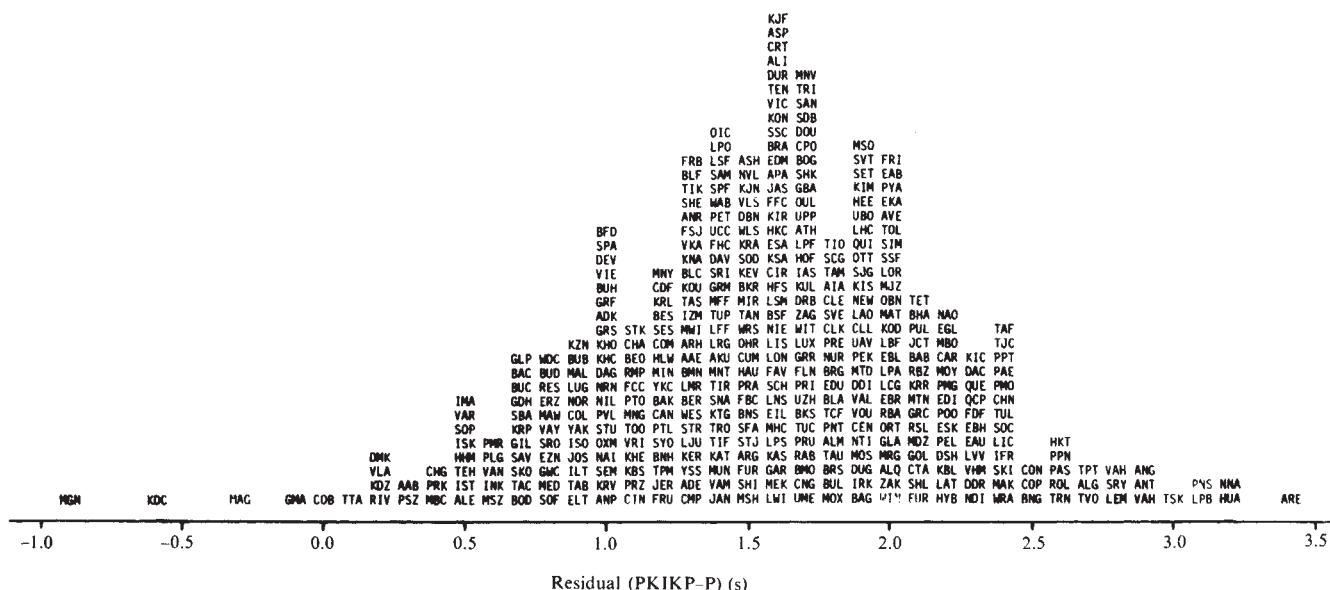


Fig. 3 Histogram of PKIKP station residuals minus P station residuals for various observatories worldwide. The observatories are given by their ISC code. A PKIKP-P residual reflects the average structure deep in the Earth beneath a seismic station.

An extended airgun array (180 m long) towed at 8 m depth was used for the measurements, with a total volume of 4,795 in², operated at a pressure of about 2,000 p.s.i. which delivered ~120 bar metres. Data were recorded to 15 s with a 60-channel streamer ~3 km long towed at 15 m depth. GECO also processed the data using conventional industrial procedures. Receiver array simulation together with the extended source had the effect of enhancing deep reflections, although at some expense to the resolution of shallow (<1–2 s) events. The data, stacked at 30-fold, are available at cost of reproduction from the Institute of Geological Sciences in Edinburgh.

Line drawings of WINCH are shown in Fig. 2 and an example of the data is shown in Fig. 3. A striking feature of the profile is that, unlike the lower crust, the upper crust over much of the line is seismically transparent. Of the major geological boundaries mapped onshore in Britain, the southerly extension of the Moine Thrust (Loch Skerrols Thrust), the Highland Boundary Fault and the Southern Uplands Fault cannot be seen. This implies that, at the scale of the wavelengths of the WINCH data (150–800 m), either these boundaries do not have a sufficient impedance contrast (change in velocity–density product), or that the boundaries are too steep to be properly imaged and there is no contrast in seismic character across them. This further implies either that at depth the geological features are insignificant or that they have died out offshore where crossed by WINCH.

The important structures of the Caledonides seen on WINCH are the Outer Isles Thrust, the Great Glen Fault Zone, the Iapetus Suture, and the South Irish Sea Lineament. The Great Glen Fault Zone (for which 2,000 km of lateral motion has been claimed³) is probably a vertical feature in the near surface consisting of at least two fault splays² inferred from reflected refractions. At depth the Great Glen Fault may extend vertically into the mantle because pronounced southerly dipping reflectors lying south of the fault zone between 10 and 13 s (~30–40 km depth) appear to be truncated (Fig. 2). Oblique reflections and offline diffractions, which are very strong in this area, complicate this interpretation and have not been removed successfully at the present stage of processing.

The Iapetus Suture (marking the position of final closure between the Baltic and North American plates towards the end of Caledonian events⁴) is not apparent in the top 3 s (uppermost 10 km). However, a pronounced change in crustal reflection character occurs between ~10 km depth and the Moho depth at 30 km, and the suture may be defined here by the north-dipping top surface of the very reflective lower crustal wedge. To the north, under the Midland Valley, few reflectors are seen but to the south the middle and lower crust of northern England contains numerous reflecting horizons.

The Outer Isles Thrust⁵ and the South Irish Sea Lineament are the only features on WINCH which can be traced as continuous reflecting zones from the surface into the lower crust. The Outer Isles Thrust (seen on two of the WINCH lines and on MOIST) dips east at 25°/–5° to depths of 18–20 km. The South Irish Sea Lineament, which outcrops to the south-west of the Llyn Peninsula, dips WNW at 25°/–5° to 15–18 km depth (using information provided by Shell Expro UK) and thus has an approximately Caledonoid trend. It is probably a thrust. We prefer to call this major basement feature the South Irish Sea Lineament rather than the more specific Menai Straits Line^{6,7} because at present we do not know exactly which onshore structure the lineament corresponds to.

Sedimentary basins are clearly imaged by WINCH when they are filled with Permo-Triassic or later rocks. They have a distinctly variable character. Some are half-grabens (for example, the Minch, Colonsay and Kish Bank Basins); others are synclinal with modest dips on the limbs (for example, 10–15° for the Stanton Trough) or steep dips (for example, up to 20° for the Cardigan Bay Basin). The North Lewis Basin and the northern end of the St Georges Channel Basin are half-grabens that lie in the hanging walls of thrusts (the Outer Isles Thrust and South Irish Sea Lineament respectively) and were clearly formed by

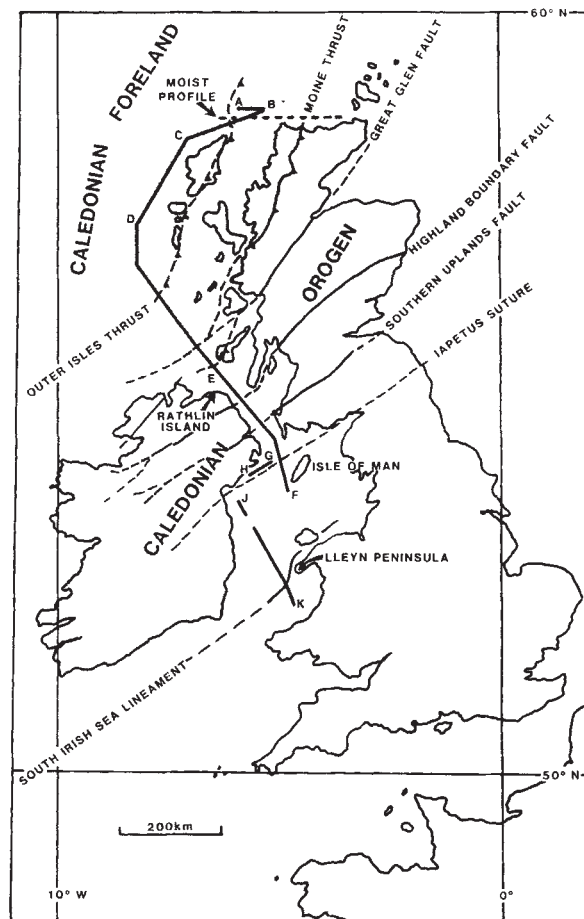


Fig. 1 Location of WINCH with respect to Caledonian foreland and orogen, and major Caledonian faults. Letters alongside profile refer to line drawing segments in Fig. 2.

the reactivation of earlier compressional features. There are other examples on the MOIST profile¹. The Solway Basin, although synclinal, also lies above the hanging-wall of the Iapetus Suture.

WINCH provides important new information on the structure and behaviour of continental crust in general. The following conclusions can be drawn from initial inspection of the data.

(1) Extensional features—several Mesozoic basins—were formed by reactivation of earlier Caledonian basement thrusts. A striking example is shown in Fig. 3. Thrusts are rejuvenated by simple normal movements on their hanging walls (such as the Minch and North Lewis Basins). A more elaborate form of rejuvenation, the 'domino' effect in which normal faults are formed by tilting and rotation of a series of subparallel earlier thrusts, is seen on MOIST along the offshore extension of the Moine Thrust Zone. Basins that formed by simple normal movements along earlier thrusts are also seen on COCORP data crossing the Coastal Plain of the eastern USA⁸. These observations complement inferences that currently active thrusts reactivate earlier normal faults along the Zagros Fold Belt⁹.

Because these basement thrusts are important to the structure and evolution of the sedimentary basins, study of the basement clearly is commercially advantageous. Mapping of basement reflectors could be carried out by the oil industry with minimal extra effort and expense if commercial seismic data were recorded to 7–10 s as a matter of course¹⁰.

(2) Acoustic boundaries on WINCH cannot be traced with certainty from the surface to the Moho or vice versa. The Outer Isles Thrust and South Irish Sea Lineament are well defined reflecting horizons in the upper crust which, however, seem to die out in the lower crust. These horizons therefore seem to be comparable with thrusts seen on COCORP data, such as the Wind River Thrust in Wyoming¹¹ and possibly the Mountain

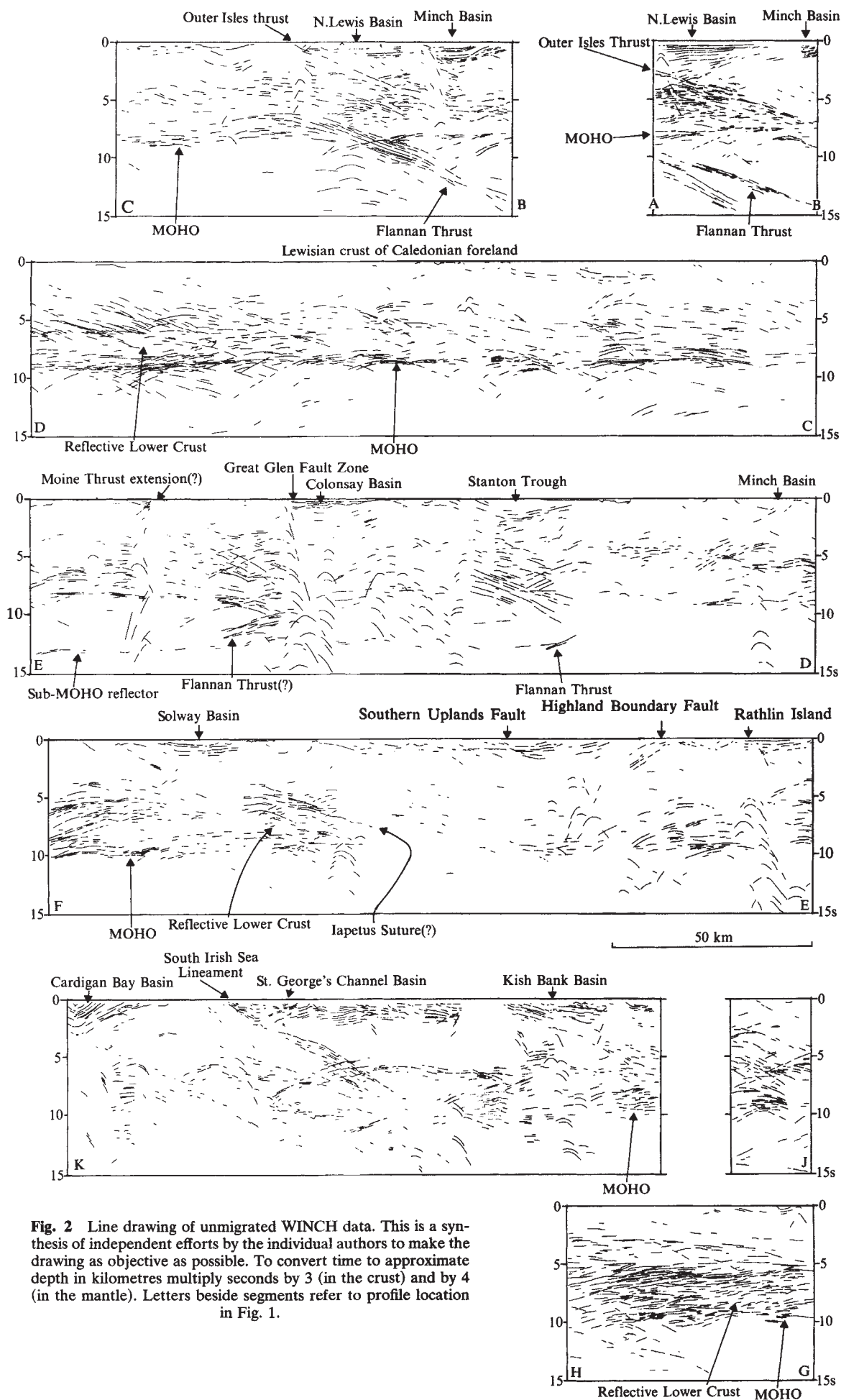


Fig. 2 Line drawing of unmigrated WINCH data. This is a synthesis of independent efforts by the individual authors to make the drawing as objective as possible. To convert time to approximate depth in kilometres multiply seconds by 3 (in the crust) and by 4 (in the mantle). Letters beside segments refer to profile location in Fig. 1.

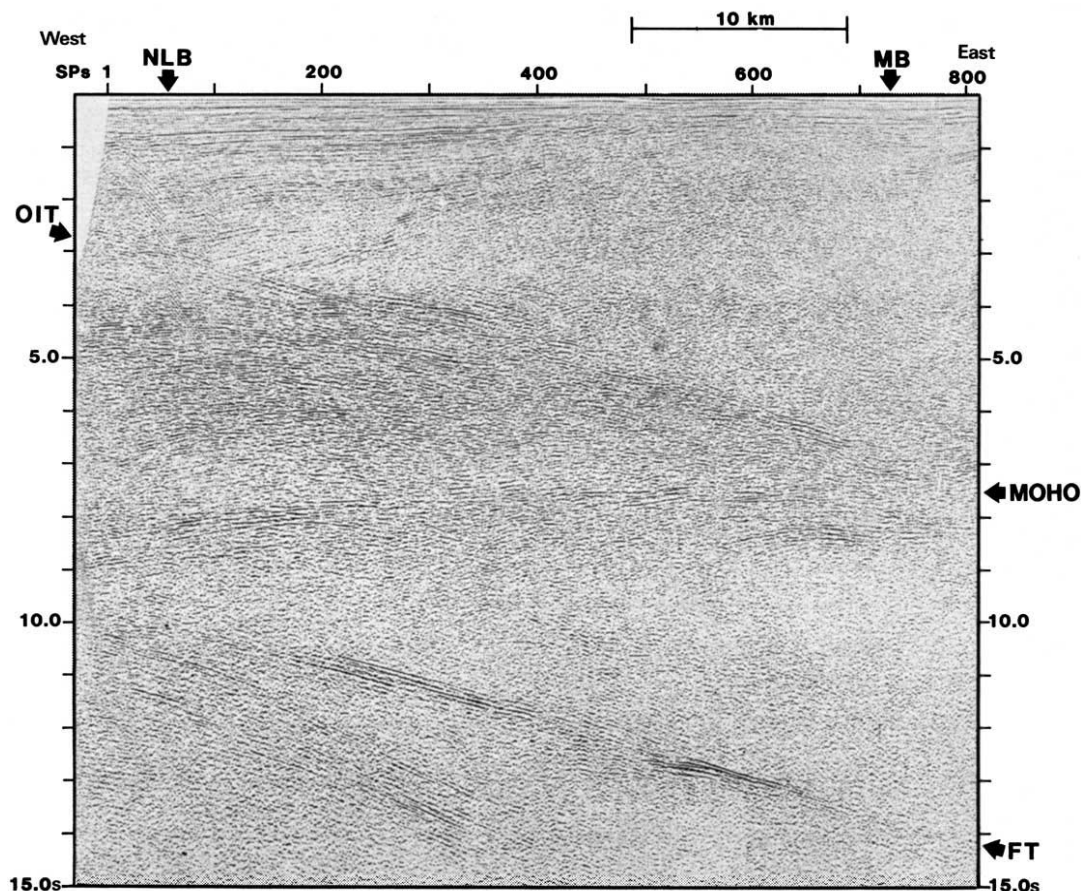


Fig. 3 WINCH unmigrated profile A-B (Fig. 1). NLB, North Lewis Basin; MB, Minch Basin; OIT, Outer Isles Thrust; FT, Flannan Thrust.

View Fault in Oklahoma¹², which also cannot be traced to the Moho. The Iapetus Suture is an example of a marked contrast of reflection character in the lower crust which cannot with confidence be traced to the surface, and the Great Glen Fault can be seen possibly in the lower crust and upper mantle as a vertical zone of reflector truncations but is poorly defined in the upper crust.

(3) The lower and middle crust has a very pronounced reflection character over much of WINCH, while the upper crust is often remarkably transparent. Antiformal reflections and diffractions that criss-cross in a complex manner in the lower crust will need careful migration to be properly resolved. With the exception of the suggested trace of the Iapetus Suture, there is little obvious correlation between variations in this lower crustal reflecting zone and changes in surface geology. Similarly reflective lower crust has been described from parts of Europe (R. Meissner, personal communication), Australia¹³ and on COCORP data in the United States¹⁴.

(4) Moho character is highly variable. Strong and fairly continuous Moho reflections are seen under the Caledonian Foreland where crystalline basement is close to the surface and Moho depth is relatively constant at 26–30 km. This character is consistent with that inferred from wide-angle reflection and refraction experiments in this area^{15,16}. Under the orogen the Moho reflection is much more discontinuous, possibly due to the complexities of geology and wave paths in the near-surface. Sometimes the Moho appears to be simply the base of the lower crustal reflecting zone (for example, in the region of the Irish Sea just south of the Iapetus Suture). Further south, under the central and southern Irish Sea, the Moho is difficult to identify. Perhaps this is related to the presence of an anomalous 7.3 km s^{-1} lower crustal layer determined by refraction experiments¹⁷. Such clear and relatively continuous Moho reflectors as are observed in the Caledonian Foreland have not yet been reported elsewhere.

(5) Strong and continuous reflectors from the mantle, which to our knowledge have also not yet been recorded elsewhere, occur

on the portion of WINCH north of Rathlin Island (Figs 1, 2). The most spectacular of these, the Flannan Thrust, lies north of the Hebrides and is seen on two WINCH lines and on MOIST¹. The seismic data show that it dips at 25–35° ENE, subparallel to the Outer Isles Thrust. The Flannan Thrust is the only reflector on WINCH which unequivocally cuts the Moho, above which it flattens in the lower and middle crust. We suspect that it may not continue to the surface. We therefore do not know the nature of this feature but suspect it is a Caledonian thrust because it has a similar attitude to the Outer Isles and Moine Thrusts.

The Flannan Thrust probably extends south of the Hebrides because a fairly weak reflector in the mantle occurs in the expected position along strike. We speculate that the Flannan Thrust is also the cause of the zone of south-dipping reflectors lying between 10 and 13 s south of the Great Glen Fault Zone (Fig. 2). If so, and if these reflectors are indeed truncated north of the fault zone, they would imply ~100–150 km of left-lateral offset along the Great Glen. A sub-horizontal mantle reflection at 35–40 km depth underlies the possible continuation of the Flannan Thrust south of the Hebrides and south of the Great Glen and may be a detachment surface into which the thrust flattens. Thus discernible seismic impedance contrasts definitely exist below the base of the Earth's crust. Clearly, 30–50 s reflection data are required to study upper mantle structures and determine the deeper extent of the Flannan Thrust.

The WINCH data are the second phase of a programme to study the deep crustal structure round the United Kingdom by seismic profiling. Detailed descriptions and analyses are in preparation. However, the data contain fascinating new information about the nature of continental crust and upper mantle. Much of the deep structure of the Caledonian orogen is resolvable and the presence of such features as the Flannan Thrust and the highly reflective lower crust need to be explained in any model of the formation of the orogen.

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while they recorded and processed the data. We also thank Shell Expro UK Ltd, especially Rodney Calvert and Bill Wheatley, for information which helped the interpretation of the data. Scientists visiting the BIRPS core group based at Bullard Labs were supported by the Department of Energy (D.K.S.) and Shell Expro UK (J.H., R.J.W.). Cambridge Earth Sciences contribution no. 420.

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Major southward thrusting of the Dalradian rocks of Connemara, western Ireland

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The Connemara schists are of Dalradian (late Precambrian–Cambrian) age and since the work of Kilburn *et al.*¹ it has been apparent that they occupy an anomalous geographical position south-southeast of the strike continuation of the Dalradian rocks of Scotland and Ireland, and south of the inferred position of the Highland Boundary Fault (Fig. 1). Also, the Connemara schists are unusual in having reached a higher metamorphic grade and having had a different metamorphic history from most other parts of the Dalradian. The model which we propose here for the Connemara Dalradian, that of an allochthonous thrust sheet, offers an explanation for these anomalies and highlights hitherto unsuspected similarities between the tectonic histories of the south-east margins of the metamorphic Caledonides in the British Isles and Scandinavia.

The Connemara schists are separated from the nearest Dalradian outcrop by the South Mayo Trough (Fig. 1) but the structural relations between the schists and the deformed early (Tremadocian) to middle Ordovician successions² in the trough are unknown, due to overlap by an unconformable Silurian (Upper Llandovery) sequence. Thus it is not clear whether: (1) the Connemara schists were close to the South Mayo Trough when it developed and filled; (2) the Dalradian and Ordovician rocks have been juxtaposed by major transcurrent movements; or (3) the rocks of the South Mayo Trough are allochthonous and have been thrust northwards over the Dalradian. Thus crustal stretching of the Dalradian could have resulted in the formation of the South Mayo Trough, with the implication of a pre-existing continuity in Dalradian structure and sequence between Mayo and Connemara, and oceanic or other material may underlie the trough.

On the south side of the Connemara massif (Fig. 2) the ~400-Myr Galway granite and Galway Bay hide the relationships of the Dalradian rocks to the folded, cleaved and horn-

felsed South Connemara Group^{3,4}, which is generally supposed, but not proven to be of Arenig age. It is agreed^{5,6}, however, that the major Skerid Rock Fault, possibly correlated with the Southern Uplands Fault (Figs 1–3), separates the Connemara massif from the South Connemara Group. In short, lack of knowledge of the relationships between the Dalradian and Ordovician rocks in the Irish equivalent to the Midland Valley of Scotland does not enable a choice to be made between possible models for explaining the anomalous position of the Connemara Dalradian.

The Dalradian rocks of Connemara have been folded by D2 folds, for example, the Derryclare (Lissoughter) fold⁷, which have nappe-like dimensions (Fig. 3). During D2 a suite of ultrabasic and gabbroic intrusions, termed the metagabbros, together with quartz diorite and other quartzofeldspathic rocks, collectively referred to as the Connemara gneisses, were intruded within an 80-km long east–west zone⁸. One of the bodies, the Cashel intrusion, has been dated at ~500 Myr by U–Pb analysis of zircons⁹. The main penetrative LS fabric in the Dalradian rocks developed during D2, with the peak of the regional metamorphism occurring between D2 and D3 (MP2) and reaching sillimanite grade in the south of the Connemara schist outcrop. Numerous D3 folds formed just after the metamorphic climax and together with the earlier fabrics, metagabbros and gneisses were refolded by the D4 Connemara Antiform (Fig. 3).

Detailed mapping by one of us (B.E.L.) south of Clifden in 1958–60 revealed a flat-lying major tectonic break or thrust⁸ between the Roundstone–Errismore metagabbro and the underlying siliceous rocks of the Delaney Dome Formation (Fig. 4). The existence of this structure was confirmed by M. D. Max (personal communication) who proposed that it be called the Mannin thrust. The thrust plane is marked by a zone of brecciated and recrystallized amphibolite up to 3 m thick which contains disrupted early folds and quartz lenses; it is best seen on south Errislannan near 650.477 (Irish National Grid). The mylonitic fabric is deformed by a crenulation cleavage which dips more steeply north than the banding. Above the thrust, the Errismore–Roundstone metagabbro has been mylonitized to form a fine-grained schistose rock up to ~250 m thick, the Ballyconneely amphibolite. The inverted nature of this basic sheet and its derivation from the original upper part of the metagabbro, first inferred by Leake from geochemical data⁸, has been confirmed (by D.S.). The Ballyconneely amphibolite contains the assemblage hornblende–albite–epidote–opaque minerals and is of lower metamorphic grade than the overlying rocks. It carries a penetrative north–northwest-trending lineation defined by aligned needles of blue–green hornblende. This lineation is seen in the more steeply dipping Connemara gneisses to the north as a quartz fibre lineation on a mylonitic foliation within the quartz diorite gneiss (Fig. 4). It appears to be later than the generally east–west trending D2 extension lineation in the Connemara schists, being of post-D3, pre-D4 age.

The original identity of the rocks beneath the Mannin thrust, the Delaney Dome Formation, has long been puzzling as the rocks lack bedding or obvious mesoscopic layering and show little variation in lithology. The presence of metabasic intrusions (Fig. 4) raised the possibility that the whole sequence might be a highly strained correlative of the quartz diorite gneiss suite. This possibility is now discounted by geochemical work (by D.S. and B.E.L.) which shows that the quartz–feldspar-bearing rocks have a markedly different major- and trace-element composition from the Connemara gneisses and are of acid volcanic origin, either rhyolites, ignimbrites or tuffs. Little-deformed rocks showing igneous phenocrysts of plagioclase and, or, K-feldspar are found at the lowest structural level at the centre of the dome and do not seem to have been metamorphosed before the episode of mylonitization. Finite strain increases irregularly upward towards the Mannin thrust (Fig. 4) and the upper part of the >250 m thick sequence consists of finely banded mylonites commonly containing phenoclasts of feldspar wrapped by quartz

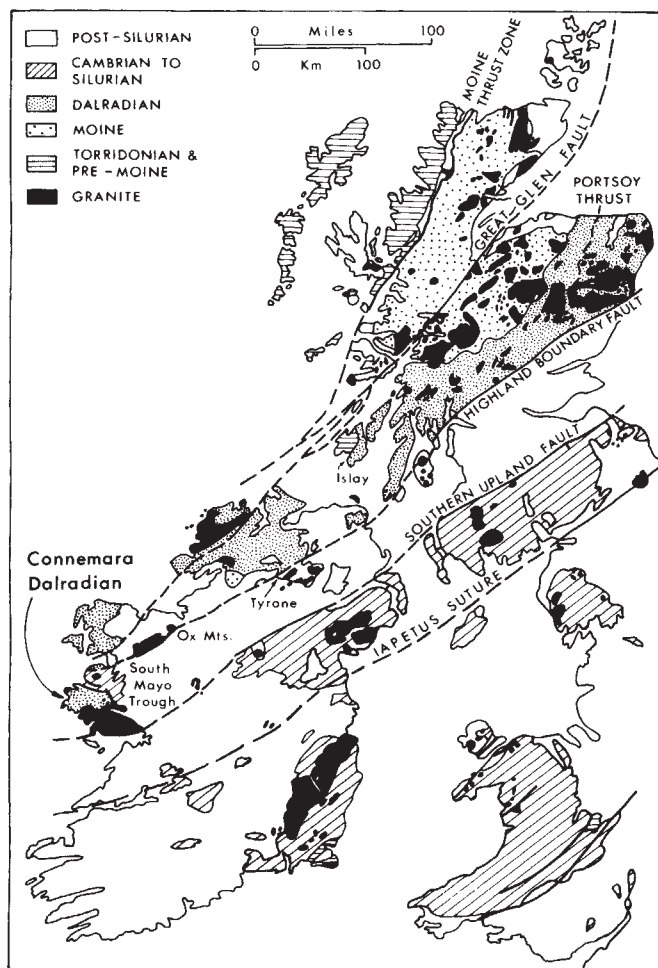


Fig. 1 Outline geological map of the British Isles showing the anomalous position of the Connemara inlier of Dalradian rocks.

folia. The bottom of the Delaney Dome Formation is not seen. All of the rocks carry a NNE–NNW-trending extension lineation marked by fibrous quartz and work is being carried out to determine the transport direction. The Delaney Dome Formation contains the assemblage quartz–albite–K-feldspar–chlorite–muscovite with some biotite and rare garnet. The garnet is of metamorphic origin and is found only in the upper, mylonitized part of the formation; the metamorphic grade reached by this part of the formation is equivalent to that of the overlying Ballyconneely amphibolite.

The Mannin thrust developed between 500 Myr, the time of intrusion of the gabbro suite, and 398 Myr, the Rb–Sr isochron age¹⁰ given by the granite which cuts the Ballyconneely amphibolite at Murvey. To constrain the age of the thrusting further, 11 rock samples of the Delaney Dome Formation have been analysed by the Rb–Sr technique (by A.N.H.) (Table 1). The isotopic data have some scatter (Fig. 5) and in determining an isochron age we have eliminated samples 40 and 42 because the feldspars are slightly reddened, suggesting hydrothermal alteration. Given these exclusions, the data scatter about a best fit line¹¹ whose slope corresponds to an age of 460 ± 25 Myr ($\pm 2\sigma$) with an initial $^{87}\text{Sr}/^{86}\text{Sr}$ of 0.7106 ± 0.0007 (SUMS 1065). All the samples were moderately to highly strained during movement on the Mannin thrust and then recrystallized with the growth of muscovite, chlorite and rare biotite. It is not certain whether this age is that of original igneous crystallization or that of mylonitization but it is well known that Rb–Sr whole rock isotopic systems in fine grained volcanic rocks are reset relatively easily¹² and further work is proceeding on this issue.

Other constraints on the ages of the main deformations in Connemara are a Rb–Sr mineral–whole rock isochron age of 460 ± 7 Myr (ref. 13) for the post-D4 Oughterard Granite and K–Ar ages on amphiboles from amphibolites in the Connemara schists and gneisses ranging from 440 (ref. 10) to 480 (ref. 14) Myr. Thus although the isotopic data do not at present define the time of thrusting precisely, it does appear that this occurred in the Ordovician between 480 and 440 Myr.

Note that the ~460 Myr (Llanvirn) age for the thrusting corresponds to a time of important crustal movements: a major

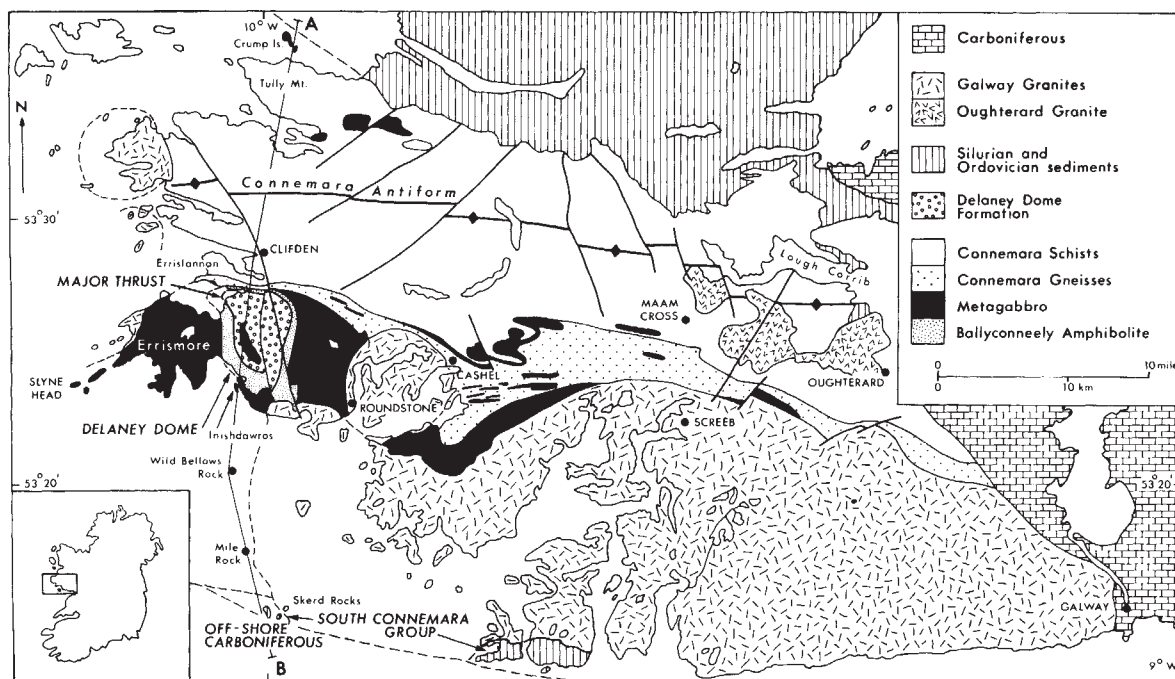


Fig. 2 Sketch map of the geology of Connemara showing the Mannin thrust, the Connemara schists, the Errismore–Roundstone ultrabasic to basic body and its tectonic derivative, the Ballyconneely amphibolite. All of these rocks overlie mylonitized acid volcanic rocks of the Delaney Dome Formation. AB is the section line for Fig. 3.

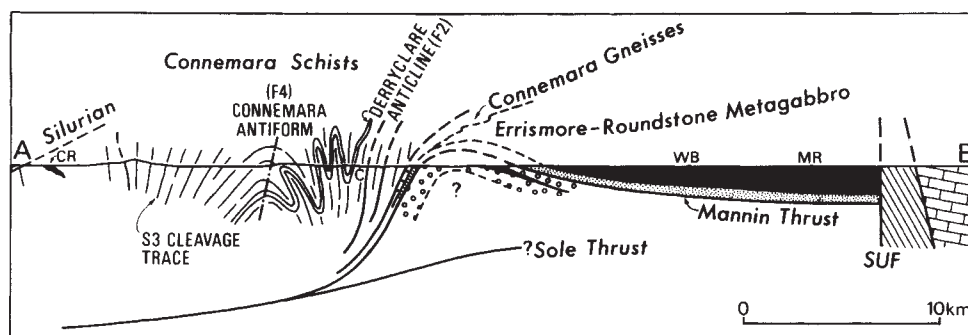


Fig. 3 A true scale north-south cross-section through Connemara from A to B (as marked on Fig. 2) showing the Delaney Dome and the Mannin thrust. C, Clifden; CR, Crump Island; MR, Mile Rock; SUF, the Skerdk Rock Fault (which may correlate with the Southern Uplands Fault); WB, Wild Bellows Rock. Key as in Fig. 2.

change in plate movements is inferred to have taken place at this time¹⁵ and it is also the approximate age of the youngest Ordovician sediments to be preserved in the South Mayo Trough and also in the Cambro-Ordovician shelf sequence of the North-West Highlands.

We conclude that the Connemara Dalradian was uplifted and thrust southwards after the intrusion of the ~500-Myr gabbro suite, either during the later stages of the D3 deformation or immediately following it. Numerous ductile thrusts or slides⁶, such as that separating the Connemara schists and Connemara gneisses in Errislunan (Fig. 4) developed both during and after D3. If thrust movements were active as late as D4 then the laterally continuous Connemara antiform may have developed above a late-formed ramp in the thrust zone. The Mannin thrust is exposed in a domal structure which has resulted from interference between a late east-west fold, the Mannin antiform and a north-south trending antiform. There is evidence of less pronounced dome and basin structures affecting the southern part of the Connemara gneiss outcrop.

During the first movement on the Mannin thrust, rocks at near sillimanite grade were thrust over cold acid volcanic rocks on the south-east foreland, resulting in retrogression of high grade rocks in the overlying slab and a prograde metamorphism of the footwall rocks. Extreme ductile deformation occurred at the base of the overthrust slab—in the Errismore-Roundstone metagabbros—and in the upper part of the underlying Delaney Dome Formation resulting in an extensive development of mylonite and a metamorphic convergence over hundreds of metres on either side of the thrust. Widespread saussuritization, sericitization and chloritization is seen in rocks for a kilometre or more above the thrust. This rapid uplift of the metamorphic core is reflected in the well-documented¹⁶ regional metamorphic growth in the Connemara schists of largely post-D3 cordierite and andalusite which represent a low-pressure assemblage ($P \approx 4$ kbar) overprinted on the earlier MS2-MP2 higher pressure (≥ 5 kbar^{16,17}) Barrovian assemblages.

In view of the recent recognition of the importance of major thrust zones, especially in the marginal parts of the Canadian Rockies¹⁸, the Appalachians¹⁹, the Variscides²⁰ and the Caledonides of Norway and Sweden²¹, it is possible that the Connemara schists and gneisses might have been carried a considerable distance to the south on a major thrust plane which could underlie most of Connemara and yet is exposed only in the Delaney Dome. The Mannin thrust zone shows features identical to those of major thrust zones found elsewhere at the margins of orogenic belts. These include the development of a prominent extension lineation transverse to the belt, the thrusting over a cold foreland of rocks still undergoing metamorphism, and the formation of a zone of domal structures along the frontal part of the belt. Therefore the Mannin thrust may be part of a major thrust zone with southerly-directed movement which developed along the south-east margin of the Dalradian rocks of Ireland in the Ordovician, possibly contemporaneous with early pre-430 Myr (ref. 22) movements on the Moine thrust zone along the north-west margin. The early tectonic transport direction in the Connemara Dalradian was possibly to the east or north-east parallel to the D2 extension lineation and possibly

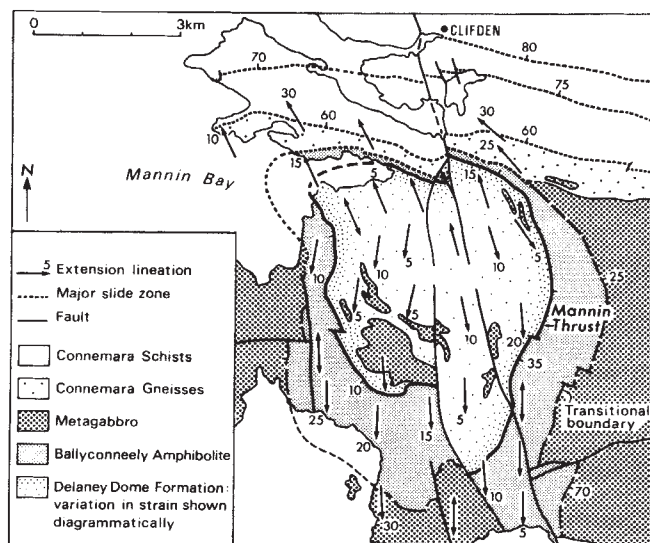


Fig. 4 Schematic map of the Mannin thrust and Delaney Dome. The variation in the trend of the lineation is due to the post-thrust folding.

Table 1 Rb-Sr isotope data on the Delaney Dome volcanic rocks

Sample no.	Rb (p.p.m.)	Rb (p.p.m.)	Rb/Sr (weight)	⁸⁷ Rb/ ⁸⁶ Sr (atom)	⁸⁷ Sr/ ⁸⁶ Sr (atomic)
40	95.55	79.54	1.201	3.486	0.73895 ± 3
42	139.5	133.1	1.048	3.039	0.73441 ± 2
136	95.06	117.2	0.8114	2.352	0.72557 ± 3
144	119.8	32.31	3.709	10.81	0.78189 ± 3
147	79.86	67.77	1.178	3.418	0.73213 ± 4
154	110.2	43.69	2.522	7.336	0.76379 ± 3
159	130.1	89.87	1.448	4.201	0.73618 ± 3
160	147.1	43.39	3.389	9.865	0.77020 ± 3
161	66.94	302.6	0.2212	0.6405	0.71490 ± 3
164	121.0	37.81	3.199	9.316	0.77354 ± 3
4,500	110.3	40.97	2.693	7.835	0.76326 ± 4

changed polarity due to a plate collision in the Llanvirn; an analogous change in polarity has been recently proposed for part of the south-east Canadian Cordillera²³.

The thrust, which may itself only be a listric splay from a deeper Sole Thrust (Fig. 3), must underlie much of the Connemara massif and since the metagabbros of the Errismore-Roundstone complex extend nearly to the Skerdk Rocks (Fig. 2), the minimum southward movement must have been over 20 km. More probably the actual movement was substantially greater and over 50 km of north-west movement is needed to restore Connemara to lie along strike from the main Dalradian outcrop of Mayo (Fig. 1). Other evidence of possible southward directed movements are seen in Tyrone where the Tay Nappe lies above

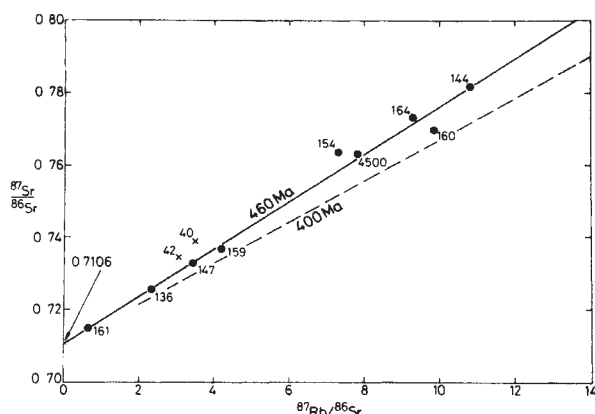


Fig. 5 Rb-Sr isochron diagram for the Delaney Dome volcanic rocks. The 400-Myr dashed line is for reference only. Samples shown by crosses are not included in the calculated age of 460 ± 25 Myr.

a major thrust plane dipping gently to the north-west²⁴ and south of Clew Bay where a recently described²⁵ northerly-dipping thrust zone brings Upper Dalradian rocks onto the Deer Park Complex.

This model could explain (1) the anomalous southern position of the Connemara Dalradian; (2) its anomalous east-west strike (being the result of possible variations in extensional strain along the strike of the thrust plane); and (3) its atypical metamorphic and structural history. The allochthonous Dalradian of the Connemara Nappe may continue as far east as Tyrone (Fig. 1) where sillimanite schists and deformed metagabbro similar to these in Connemara have been reported²⁶.

The British Isles segment of the metamorphic 'Caledonides' could therefore have the same two-sided thrust geometry as the Greenland-Scandinavia segment, though the nappes and thrusts in the latter are generally of younger, Silurian, age. There is also some evidence that in Scandinavia at least, southward-directed thrusting and nappe emplacement began in the Ordovician²⁷. Our interpretation would remove the previously enigmatic absence of thrust structures equivalent to those found throughout the whole of the south-east Caledonide front in Scandinavia and has important implications for the pre-Silurian structure of the Midland Valley region of Scotland.

Lithologies similar to the acid volcanic rocks (Delaney Dome Formation) which underlie the Mannin thrust are virtually unknown in the Dalradian succession and their unmetamorphosed condition before mylonitization is strong evidence that they are probably of Ordovician age. They could be correlated with the mid-Ordovician ignimbrites²⁸ of the South Mayo Trough or alternatively with the acid volcanic rocks of Ordovician age which are widespread in the Lake District and North Wales. The Ordovician faunas of the South Mayo Trough have American affinities, compare well with those at Girvan (G.B. Curry, personal communication) and indicate that these rocks were deposited on the north side of the Iapetus Ocean. Correlation of the Delaney Dome rocks with an extension of the Anglo-Welsh area, carries the obvious implication that the Iapetus Suture in western Ireland, whose position is unknown at present, would lie north of Connemara.

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Regional sea level, Southern Oscillation and beach change, New South Wales, Australia

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Coastal erosion is a problem of increasing concern that affects 60% of the world's sandy coastline¹. This erosion has been attributed to increased storminess, tectonic subsidence, eustatic sea-level rise, decreased shoreward sediment movement from the shelf, permanent longshore leakage of sediment from beach compartments, shifts in global pressure belts resulting in changes in the directional component of wave climates, and human interference^{2,3}. No one explanation has worldwide applicability because all factors vary in importance regionally. Evaluation of factors is complicated by a lack of accurate, continuous, long-term erosional data. Historical map evidence spanning 100-1,000 yr has been used in a few isolated areas⁴⁻⁷; however, temporal resolution has not been sufficient to evaluate the effect of climatic variables. Air photographic evidence⁸ is restricted to the past 40 yr, and often suffers from insufficient ground control for accurate mapping over time. Ground surveying of beaches was rarely carried out before 1960 and is often discontinuous in time and space. I have resolved the problems of temporal and spatial continuity by studying change for the whole of Stanwell Park beach, New South Wales, Australia for the period 1895-1980 (Fig. 1). I report here that using the average high-tide wave run-up position measured accurate to ± 2.5 m from oblique and vertical photographs, changes could be linked to regional sea-level variation and a globally significant climatic variable, the Southern Oscillation (SO).

Stanwell Park beach, located on the tectonically-stable South Coast of New South Wales¹², is a compartmentalized, exposed, ocean beach having no permanent longshore leakage of sediment and little human interference. The beach faces the main south-east swell which averages 10 s in period and 1.2 m in wave height⁹; however, the beach is modified to some degree by all wave directions affecting this coastline³. Inshore topography varies rapidly from alternating shore-tied shoals and rip channels to a single, shore-parallel bar-trough in response to storm waves which often exceed 4 m in height.

Oblique tourist photographs, dated 1895-1980, were solicited from the public using local and national newspaper advertisements, and were supplemented by photographs from historical societies, government departments, postcard companies and libraries. Vertical photographs taken by state and federal governments since 1948 were also collected. Undated photographs were sequentially ordered and dated to the nearest year using geomorphic, vegetational and accurately dated cultural changes. Shadow positions of telegraph poles and trees of known elevation were then used to calculate solar azimuth, elevation and hour angle¹⁰. From this information the exact time of year could be obtained with spring and autumn months

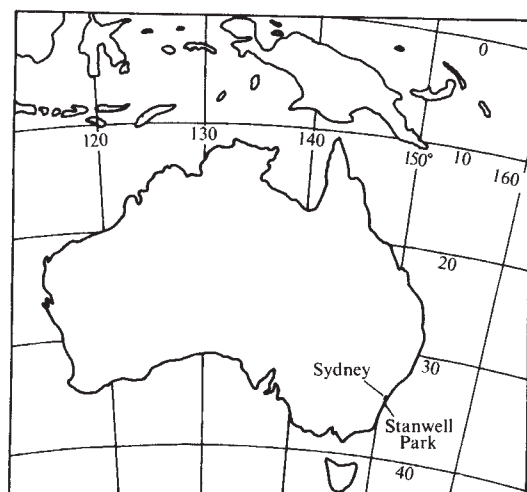


Fig. 1 Location map of the study area.

Table 1 Correlation of high-tide position with climatic factors between 1895 and 1980

	SO index (quarterly 1897–1974)	Sea level (monthly 1897–1980)	Hadley cell position (monthly 1941–77)	Sea-surface temperature (monthly 1967–76)
Time	$r = 0.117^*$ 0.013 yr^{-1} $n = 312$	$r = 0.253^{\dagger}$ 0.64 mm yr^{-1} $n = 1,020$	$r = 0.087^{\ddagger}$ $0.039^{\circ} \text{ S yr}^{-1}$ $n = 444$	NS
Mean high-tide position	$r = -0.192^*$ $n = 117$	$r = -0.194^*$ $n = 128$	NS	NS

Pearson product-moment correlation coefficients between high-tide wave run-up position and SO index, Sydney sea level, Hadley cell position on East Coast of Australia, and sea-surface temperature for 1° square $33^{\circ}\text{--}34^{\circ}\text{S}$, $153^{\circ}\text{--}154^{\circ}\text{E}$.

* Significant 0.05 level. † Significant 0.01 level. ‡ Significant 0.10 level. NS, not significant.

being separated on the basis of vegetational differences. The record of 128 photographs averaged one every 4 yr between 1895 and 1920, one every 2 yr between 1920 and 1933, and more than 1 per yr thereafter.

A direct linear transformation method¹¹ requiring a minimum of six control points was used to transform the position of high-tide wave run-up from single photographs to plan maps. Single rather than stereo photographs could be used because ground surveying over time indicated that high-tide run-up was stable at 3.5 m above low tide (standard error, 0.56 m). The accuracy of the method was evaluated further by comparing plan maps of identical photographs analysed more than once, and of photographs taken at the same time from different locations or using different cameras. Total mapping error amounted to ± 2.5 m. Ninety-five per cent of photographs were analysed using seven or more control points with a standard error of <1.5 m.

Deviations from mean high-tide position (Fig. 2) show that, apart from the 1-in-100 yr storms of 1974, the beach has undergone continuous and constant fluctuations in the magnitude of erosion and accretion since 1930 with no significant loss or gain of sediment since 1895³. In the 1930s and 1940s changes were superimposed on an accretional trend. Beginning in 1948 coastal recession has predominated except for the period 1970–74. Between 1895 and 1967 erosion on Stanwell Park did not always occur contemporaneously with documented erosion on adjacent beaches in the region¹². Discrepancies can be attributed to the sporadic nature of documentation along the New South Wales coast and its bias towards the destruction of manmade structures infringing on the active coastal zone—an

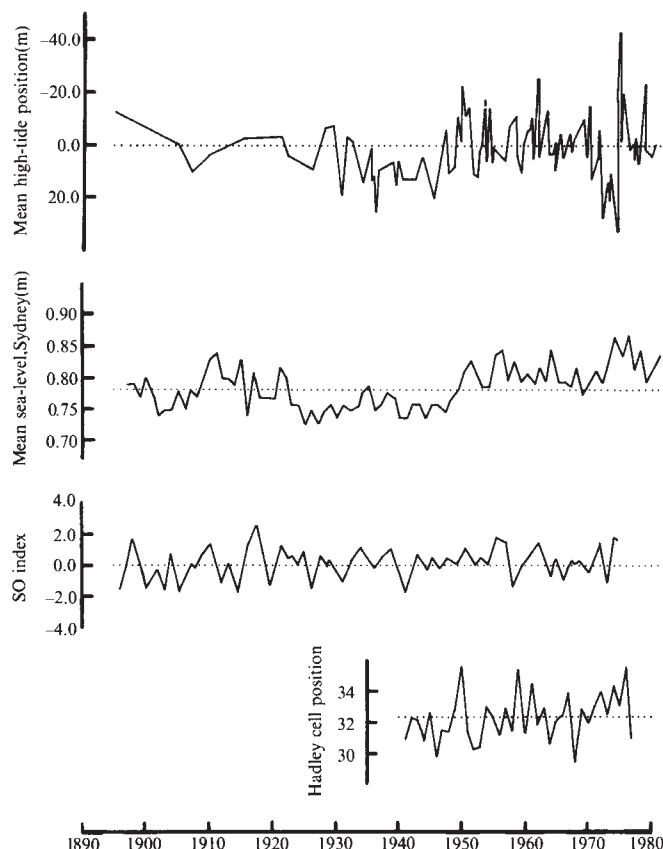


Fig. 2 Changes for various periods 1895–1980 in high-tide wave run-up position averaged for whole of Stanwell Park beach (values measure deviations from mean record), sea level at Sydney, Southern Oscillation index (values measure the normalized pressure difference between Darwin and Tahiti), and latitude of the centre of the Hadley cell along the East Coast of Australia.

aspect not applicable to Stanwell Park. Since 1967 when more intense coastal research commenced in New South Wales, the Stanwell Park and regional records coincide.

The date 1948 represents the beginning of a cold phase of temperature in the Northern Hemisphere^{13,14} and one of increased storminess along the US Atlantic seaboard¹⁵. In Australia this date is perceived as a climatic turning point. Autumn temperature¹⁶ and rainfall^{17–19} have increased with more frequent blocking at mid-latitudes, fewer anticyclones and poleward movement of westerlies²⁰. Responses to these changes in New South Wales include increased flood frequency on the Hawkesbury River, higher lake levels in Lake George, and channel expansion in the Hunter Valley^{21,22}. To account for the variation in coastal change, the high-tide data for Stanwell Park were correlated to monthly mean sea level measured at Sydney 40 km northwards, the quarterly barometric pressure difference between Darwin and Tahiti²³, the monthly position of the centre of the Hadley high-pressure cell along the east Australian coast²⁴, and monthly sea-surface temperature averaged for the 1° square $33^{\circ}\text{--}34^{\circ}\text{S}$, $153^{\circ}\text{--}154^{\circ}\text{E}$ ²⁵ (Fig. 2, Table 1). Additionally, these meteorological and oceanographic variables were lagged and then linearly cross-correlated with and without seasonal effects (Fig. 3).

Each of these variables has direct relevance to coastal erosion. Sea-level variation has been related to erosion on beaches having equilibrium profiles and little longshore sediment movement^{1,26}. In these conditions, as sea level rises, sediment moves offshore to maintain a uniform profile shape and the shoreline retreats landward. The process is known as Bruun's rule and has been invoked to account for erosion rates of 0.21 and

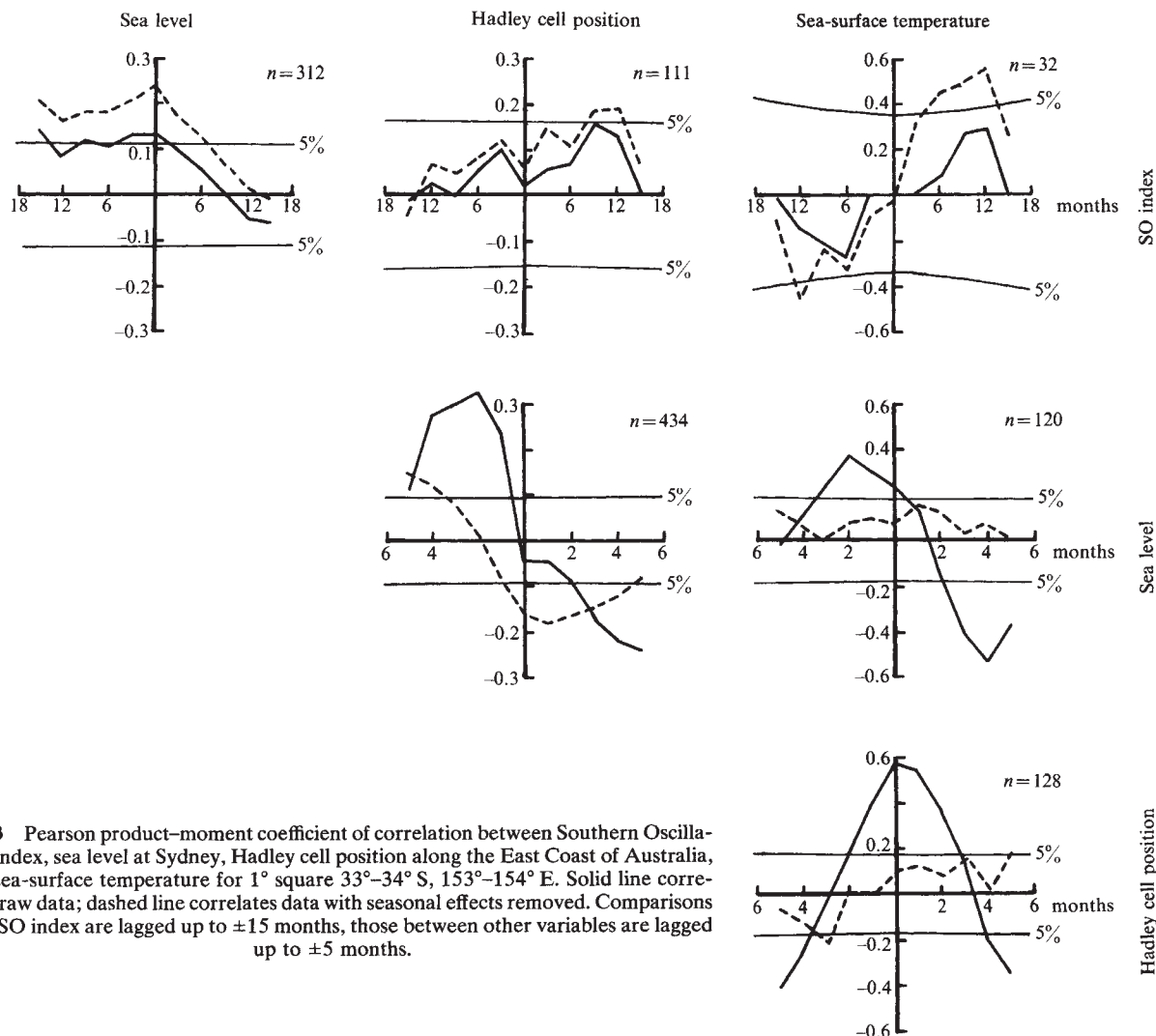


Fig. 3 Pearson product-moment coefficient of correlation between Southern Oscillation index, sea level at Sydney, Hadley cell position along the East Coast of Australia, and sea-surface temperature for 1° square 33° – 34° S, 153° – 154° E. Solid line correlates raw data; dashed line correlates data with seasonal effects removed. Comparisons with SO index are lagged up to ± 15 months, those between other variables are lagged up to ± 5 months.

1.81 m per cm rise in waterlevel per yr in the Great Lakes and Chesapeake Bay respectively^{27,28}. Sea-level change also affects beach water tables. Measurements taken over a tidal cycle^{29,30} and over a 5-yr period at Warilla beach 30-km south of the study area³¹, together with experiments in which water tables were raised artificially³², have shown that substantial beach erosion can be initiated by rising water tables associated with rising sea level.

The Darwin–Tahiti pressure difference values, normalized here to a standard deviation of 1.0, measure the strength of a longitudinal pressure wave across the Pacific Ocean—Walker circulation. This circulation is inherently linked out of phase to sea-surface temperature in the East Pacific³³. The phenomenon, also known as the SO, oscillates every 2–3 yr and, at times of warmest sea-surface temperature—the El Niño of Peru—has been related to a wide range of climatic events worldwide including equatorward displacement of subpolar lows and subtropical highs, below average monsoonal rainfall in India, drought in Australia and colder winter temperatures in the United States³³. Regionally the Hadley cell positioning along the east coast of Australia controls the strength and persistence of erosive onshore winds. A southward movement has been correlated to increased rainfall in New South Wales¹⁷. Enhancement of east–west flow caused by southward movement of the Hadley cell beyond 40° S, coupled with increased sea-surface temperature anomalies, has been envisaged as increasing the intensity and duration of cyclogenesis (storms), especially in autumn, along the New South Wales coast³⁴.

Overall the SO index and regional sea level have increased (0.05 level of significance) since 1897 while the centre of the

Hadley cell has shifted (0.10 level of significance) southwards by 1.4° since 1940. Sea-level rise has amounted to 0.64 mm yr^{-1} which is less than the eustatic rate of 0.95 mm yr^{-1} (ref. 35). Specifically sea level fell or was stable between 1910 and 1946 during the main accretional phase on Stanwell Park beach and rose in a step-functional manner by 5–10 cm after 1948 concomitantly with beach recession. Oscillations before 1950 are not coincident with world-scale climatically induced fluctuations in eustatic sea level³⁵—a fact suggesting that regional long-term variations in meteorological variables such as barometric pressure, the incidence of storm surges or frequency of induced shelf waves are more important in controlling the Sydney sea-level curve^{36,37}. Both the SO index and sea-level correlate inversely (0.05 level of significance) with beach accretion ($r = -0.192$ and -0.194 respectively). The Hadley cell position and sea temperature appear to have no such effect on beach position. Increased hemispheric easterly flow (positive SO index) also results both seasonally and over the long-term in a rise in sea level ($r > 0.138$). While Hadley cell movement is related to the SO index in that it precedes the index by 9–12 months ($r > 0.153$), it does not affect shoreline change. Seasonally southward movement of the Hadley cell increases sea-surface temperature ($r = 0.542$) and, after a three month lag, sea level ($r = 0.324$). These increases are directly attributable to an associated poleward shift in the warm East Australian current. The long-term poleward displacement of the Hadley cell produces decreased sea level—a result that does not facilitate beach recession. Isolated movement and stagnation of the Hadley cell poleward of 40° S in autumn has exacerbated erosion only for the storm periods of 1949, 1956 and 1974.

This erosion may be associated with increased cyclogenesis and higher sea-surface temperatures, but the sea-temperature record, short though it may be, indicates that beach recession has followed periods of both high and low sea temperature.

Long-term coastline recession and accretion on Stanwell Park beach are linked primarily to variations in sea level produced by phenomena associated with fluctuation in the Walker circulation. Increased storminess, or factors responsible for storms are not as important, as evidenced by the fact that the magnitude and frequency of erosional events has remained constant since 1930 during both accretional and erosional phases. While sea-

level fluctuations have not exceeded 12 cm, a 1-cm rise in sea level can be related significantly to a 0.5-m recession of the high-tide line. This rate is similar to those reported elsewhere^{27,28} invoking Bruun's rule to account for shoreline erosion. Recession must also be aided by an associated rise in beach water tables. The fact that a hemispheric climatic variable, which can be linked to a wide range of climatic events worldwide, is also linked regionally to sea-level variation and beach recession on Stanwell Park beach, may warrant a change in emphasis to world climatic variability as a major cause of present day coastal erosion.

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Fly-ash particles intercepted in the deep Sargasso Sea

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Results of a continuing series of sequential sediment-trap deployments in the Sargasso Sea, covering 5 yr, suggest that an efficient mechanism exists for the removal of both biogenic and abiogenic particles from the surface layers of the ocean and for their rapid transfer to great depth^{1,2}. We report here the occurrence of fly-ash particles in material collected in that experiment. Available evidence indicates that these particles were carried by winds from their sources, probably located in North America, to the Sargasso Sea and subsequently transported to deep water as components of faecal pellets or other organic aggregates. The speed of their removal from the water column suggests that their geographical distribution on the sea floor is dictated mainly by their airborne transport. The conspicuous morphology and specific chemistry and mineralogy of fly-ash particles may make them useful indicators of the presence of industrial pollutants in remote parts of the ocean. As their production is mainly limited to the past 100 yr, their appearance in sediments may serve as a time marker or as a tracer for biological down-mixing of particles.

Samples were collected in a sediment trap of 1.5 m² cross-section, moored 3,200 m below the surface and 1,000 m above the sea floor, ~45 km south-east of Bermuda. Details have been given previously¹. Earlier studies of the samples showed that the flux of particles into the trap varied in phase with the annual cycle of primary production in the surface water³. Even

the <37- μ m fraction (as obtained by passing the bulk material collected through a set of sieves) exhibited this seasonal variation which can be maintained at that depth only if the sinking time is short. Electron microscopic investigations of the samples showed land-derived abiogenic particles among predominantly biogenic material. Remarkable among these are fly-ash spherules.

Fly-ash particles of the type encountered in our samples originate in coal-burning power plants⁴, of which there are none on the island of Bermuda. The size distribution, chemistry and mineralogy of such particles at the source have recently been studied in detail^{4,5}. Common features of fly-ash particles are their spherical shape, glassy texture and chemical composition. They acquire their shape during cooling of the non-combustible mineral content of coal, melted at temperatures in excess of 1,500 °C, while being carried along by the gaseous combustion products. The cooling may be sufficiently slow to allow the formation of frameworks of acicular mullite crystals embedded in a glassy matrix⁴. The framework, usually hidden under an amorphous surface layer, is easily exposed by hydrofluoric acid leaching. Particles with an amorphous layer contain more metallic constituents and have higher Si contents than those without; they also have highly variable Al/Si ratios. The crystalline particles have high, less variable, Al/Si ratios and fewer constituents.

Figure 1 shows typical fly-ash particles observed in the sediment-trap samples. They are embedded in siliceous biogenic debris and clay aggregates. In contrast to fly-ash particles collected from power plants, which reveal their acicular framework only after HF leaching, those encountered in the sediment-trap samples appear to exhibit the framework in their natural state or after no more than HCl leaching. They appear to be rare in samples examined before HCl treatment. Sub-milligram samples of the <37- μ m fraction of the dried bulk sediment contained an average of 10 particles in the 10-15 μ m size range. However, our observations are not yet sufficiently quantitative to allow flux calculations. Acid leaching led to relative enrichment of the fly-ash particles by dissolving the calcareous debris which constituted 45-60% of that size fraction.

The spherical particles were found only in sizes of <15 μ m diameter. Such a size range is similar to that of power plant

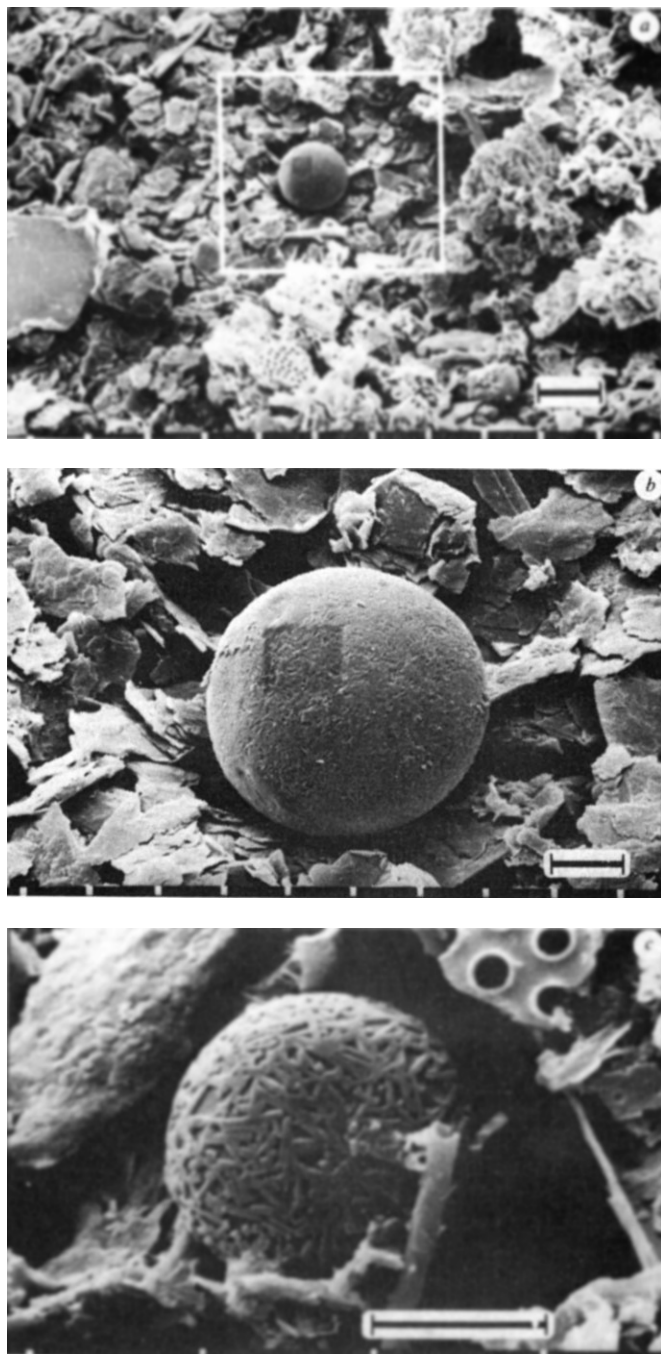


Fig. 1 Scanning electron micrographs of residue of sediment-trap samples treated with HCl to remove carbonate: *a*, fly-ash particle embedded in biogenic debris and clay minerals; *b*, close-up of particle shown in *a* (dark square shows region analysed by EDAX); *c*, particle with well-developed mullite framework. All micrographs taken of gold-coated samples at 8-kV accelerating voltage. Scale bars: *a*, 10 µm; *b*, *c*, 3 µm.

emissions which is largely between 0.05 and 20 µm (ref. 5). The lower cutoff at the high end is probably due to settling of the larger particles closer to source areas, which in this case must be more than 1,000 km away. Their transport to Bermuda from North America is in keeping with recently observed evidence for the arrival there of other airborne pollutants^{6,7}.

Energy-dispersive X-ray analysis (EDAX) showed that the particles from the sediment trap have very similar major element (Al, Si, K) distributions (Fig. 2*a*). The particles are poor in Ni. This distinguishes them from spherules found in deep-sea sediments which are thought to be of extraterrestrial origin^{8,9}.

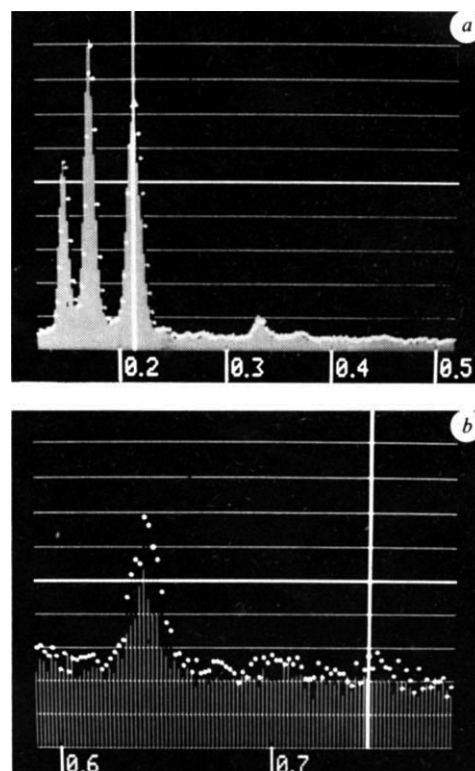


Fig. 2 Superimposed EDAX spectra of particles shown in Fig. 1*b* and *c* (*b*, bars; *c*, dots). *a*, Major-element peaks, left to right: Al, Si, Au (from coating), K. *b*, Minor-element peaks Fe (left) and Ni (at position of white line). EDAX analyses on gold-coated samples at 20-kV accelerating voltage, 30° surface tilt and 25° take-off angle.

Spherules of the size range observed in the sediment-trap samples are commonly-observed constituents of both filtered air and filtered surface seawater samples of the North Atlantic¹⁰⁻¹² and elsewhere¹³. Few systematic studies of these particles have yet been made although industrial, extraterrestrial and volcanic origins have been suggested for certain groups¹⁰⁻¹⁶. If fly-ash particles are as abundant off-shore as some marine sampling programmes suggest, then the relative ease with which they can be recognized should make them useful tracers of anthropogenic input to sediments in even remote parts of the ocean. They may serve as historical markers in a manner analogous to that suggested for elemental carbon particles in aquatic environments^{17,18} or as tracers of the down-mixing of sediment particles by benthic organisms. In the latter application they are clearly superior to many chemical tracers which undergo various transformations after deposition.

The occurrence of the fly-ash particles in our samples in association with clay aggregates embedded in biogenic debris suggests that they were transferred from the surface to the deep water in a mode analogous to that suggested earlier for the clay particles². That transfer involves removal from the surface water through their uptake by organisms and incorporation into larger, rapidly sinking faecal pellets and other organic aggregates which, in turn, may sweep up additional particles during their descent. Rapid sinking limits horizontal transport within the ocean. The fact that some of the fly-ash particles collected in the sediment trap exhibit their acicular framework may be due to the removal of the heavy-metal enriched amorphous layer hiding this framework during transport. The layer may have been leached away within the guts of organisms responsible for their incorporation into the larger aggregates. The association of the fly ash with the clay helps confirm the atmospheric input of clay to the Sargasso Sea.

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Nonconstant extinction rates of Neogene planktonic foraminifera

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Van Valen's 'new evolutionary law'¹ has provoked considerable discussion of survivorship of fossil taxa^{2–5} and of the Red Queen's hypothesis^{6,7}. Van Valen's principal conclusion was that survivorship curves of fossil taxa are essentially linear¹, indicating that the mean probability of extinction within an ecologically homogeneous group has been constant^{1,8}. Despite some criticism about his methodology^{9–11}, constant extinction rates have been regarded as the general rule for fossil taxa¹² and the empirical basis for the Red Queen's hypothesis^{7,13,14}. We show here the fallacy of the concept of constant extinction rates by applying his survivorship-curve technique^{1,2} to longevity data for Neogene planktonic foraminifera¹⁵ which clearly exhibit nonconstant extinction rates through geological time. The linearity of survivorship curves only indicates that the probability of extinction of a species is independent of its longevity. The mean longevities and extinction rates of tropical, transitional and temperate assemblages are statistically similar.

The present study is based on the range charts of 148 species/subspecies compiled by Kennett and Srinivasan in their phylogenetic synthesis of Neogene planktonic foraminifera¹⁵. The appearances/disappearances of these species have been correlated to the radiometric time scale. We have only considered the 94 extinct species contained in this data set to avoid creating artificial extinctions for living forms. The exclusion of living species would create a small bias⁸. The extinct species have been subdivided into tropical, transitional and temperate categories according to their mean geographical distributions for comparison of survivorship curves in different climatic regimes. The extinctions considered include phyletic extinction (pseudoextinction) and termination extinction. The ratio of pseudoextinction to total extinction is about 0.38. We have used the species durations in millions of years as the abscissa and plotted the surviving frequency distributions of planktonic foraminiferal species against a logarithmic ordinate (Fig. 1).

For testing the linearity of survivorship curves, Raup² has recommended the use of Epstein's 'total-life method' as a more dependable method than others such as the Kolmogorov-Smirnov test. The 'total life' of a species is the sum of the durations of all species in the group before the extinction of this given species. Thus if there are n species, with durations $d_1 < d_2 < \dots < d_n$, then the total lives (T_i) are:

$$\begin{aligned} T_1 &= nd_1 \\ T_2 &= d_1 + (n-1)d_2 \\ &\vdots \\ T_i &= d_1 + d_2 + \dots + (n-i+1)d_i \\ &\vdots \\ T_n &= d_1 + d_2 + \dots + d_n \end{aligned} \quad (1)$$

In a situation equivalent to linear survivorship, the sum of the first $n-1$ total lives ($T_1 + T_2 + \dots + T_{n-1}$) is normally distributed with a theoretical mean of $(n-1)T_n$ and a standard deviation of $[(n-1)T_n^2/12]^{1/2}$. In testing for linearity of survivorship, $\sum_{i=1}^{n-1} T_i$ is compared with the theoretical range which is the mean $\pm 1.96 \times$ standard deviation. If $\sum_{i=1}^{n-1} T_i$ is within this range, the hypothesis of linear survivorship is accepted at the 0.05 significance level.

We have applied the total-life method to the total species as well as to those for three climatic regimes (Table 1). All four survivorship curves shown in Fig. 1 are considered to be linear at the 0.05 significance level. Thus the survivorship curves for Neogene planktonic foraminiferal assemblages again apparently prove Van Valen's law.

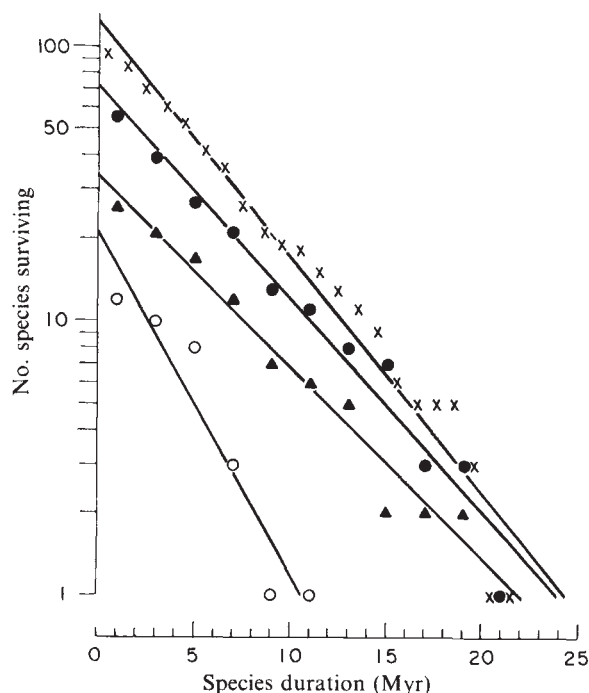


Fig. 1 Survivorship curves of total (x) Neogene planktonic foraminiferal species; and subdivisions of tropical (●), transitional (▲) and temperate (○) assemblages. Note that all taxa are treated as if they were present from the beginning and the surviving numbers of species are plotted against the logarithmic ordinate; the abscissa presents the durations of species rather than geological time. Because of the cumulative nature of survivorship curves, the straight lines determined by simple regression serve only for estimating slopes, and not for testing the linearity of survivorship curves. The linearity is tested using Epstein's 'total-life method'² (see the text). All four survivorship curves are considered to be linear within a significance level of 0.05 (see Table 1).

Table 1 Statistics for the test of linearity of survivorship curves based on the total-life method² for Neogene planktonic foraminiferal assemblages

Assemblage	Theoretical mean	Theoretical s.d.	$\sum_{i=1}^{n-1} T_i$	0.95 confidence interval
Total	26,128.3	1,564.3	28,822.1 ($z = 1.72$)	23,062.4– 29,194.3
Tropical	9,003.5	700.9	9,302.2 ($z = 0.43$)	7,629.7– 10,377.3
Transitional	2,220.0	256.3	2,640.2 ($z = 1.64$)	1,717.6– 2,722.4
Temperate	313.0	54.5	386.4 ($z = 1.35$)	206.2– 419.7

Nevertheless, we have analysed the same large body of data¹⁵ using the following approach, the details of which are provided in another paper¹⁶. We have defined the extinction rate for a time interval Δt as

$$R_e = \frac{1}{D} \times \frac{E}{\Delta t} \quad (2)$$

where Δt is the time lapse for each time interval, E the total number of extinctions within this interval and D the total number of species existing in this interval. Using this equation with $\Delta t = 1$ Myr, we have calculated the *per capita* extinction rates during the past 25 Myr. This analysis clearly shows that extinction rates were not constant but varied from 0.0 to 0.42 with respect to geological time since the latest Oligocene (Fig. 2). The fluctuations in extinction rates are considered to be controlled by diversity-dependent mechanisms and various palaeoceanographic changes that occurred during the Neogene¹⁶.

The data base for these two approaches are slightly different since the analysis of extinction (Fig. 2) includes the species that are still living for the diversity term (D) in equation (2). Nevertheless, we feel that the results are comparable. Van Valen¹ noted variability of extinction rates over geological time and stated explicitly: "The constancy of extinction in terms of survivorship does not imply constancy over geologic time and vice versa". He thus attempted to explain the linearity of

survivorship curve considering that "Extinction rates vary over time but can nevertheless approximate a distribution which is stationary over time"¹⁸. He explained this apparent equilibrium as due to various randomly acting biotic processes that regulate fitness within the adaptive zones or community of each general group, and formulated his law, that the effective environment of the members of any homogeneous group of organisms deteriorates at a stochastically constant rate¹. As an explanation, Red Queen's hypothesis states: "that the sum of absolute fitnesses in a community is constant"¹⁴. Van Valen¹ regarded small, regular and frequent perturbations in physical environment as a constant factor affecting extinction, while those massive extinctions caused by major environmental perturbations (for example Permo-Triassic Event, Terminal Cretaceous Event) differ from the 'usual' extinction process. However, the major problem is, as McCune⁵ points out, that constant extinction rates cannot be deduced from Van Valen's survivorship curves even if the curves are linear. This is because the abscissa in the plots represent the durations of taxa rather than geological time, and the durations of taxa within a group are aggregated together with no regard to their chronological positions. Van Valen and his followers have incorrectly interpreted linearity of survivorship curves as if the taxa had all originated simultaneously as in a cohort survivorship analysis. The linearity of the survivorship curves only indicates that longer-lived taxa are not necessarily more resistant to extinction than shorter-lived taxa. The different results obtained from the two approaches suggest that the vulnerability of species to extinction in response to either environmental perturbation or to stochastically diversity-dependent regulations is independent of their longevities.

However, Van Valen's survivorship curve technique is valuable in revealing some general characteristics of Neogene planktonic foraminiferal evolution. Raup² and Van Valen⁸ have made an analogy between linear taxonomic survivorship and the decay curve of radioactive isotopes defined by

$$S_t = S_0 e^{-\lambda t} \quad (3)$$

where S_t is the number of taxa for which longevity is longer than t , S_0 the total number of taxa, λ the taxonomic extinction rate per unit time which can be obtained by estimating the slope of survivorship curve with a simple regression and e the base of the natural logarithm. This implies that all taxa are treated as if they were present from the beginning, like the

Fig. 2 *Per capita* extinction rates of total Neogene planktonic foraminiferal species; and subdivisions of tropical, transitional and temperate assemblages for the past 25 Myr (latest Oligocene to Recent). Shown are Neogene (N) planktonic foraminiferal zones of Blow¹⁸, as modified by Srinivasan and Kennett¹⁹.

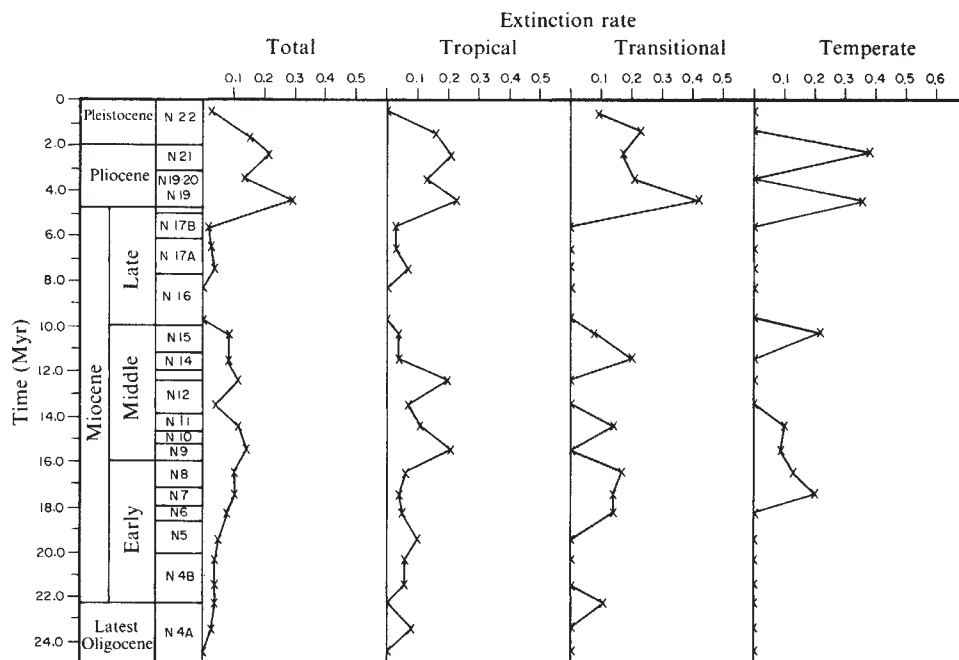


Table 2 Pooled extinction rates and mean longevity for total Neogene planktonic foraminiferal species and various assemblages

Assemblage	Extinction rate (ma)	Longevity (Myr)
Total	144 ± 7	6.0 ± 5.1
Tropical	130 ± 8	5.9 ± 5.4
Transitional	116 ± 8	6.8 ± 5.1
Temperate	209 ± 32	4.7 ± 3.1
Warm (tropical and warm subtropical)	134 ± 6	6.1 ± 5.4
Cool (temperate and cool subtropical)	147 ± 12	5.7 ± 4.0

Assemblages include subdivisions of tropical, transitional and temperate assemblage, as well as a more general warm assemblage (tropical plus warm subtropical) and a more general cool assemblage (temperate plus cool subtropical). Values are mean ± s.d.

parental element in a radioactive decay process. Van Valen¹ has defined a macarthur (ma) as the unit of extinction rate which yields a half life of 500 yr:

$$1 \text{ ma} = \frac{-\lambda}{\ln 0.5} \times 500 = \frac{\lambda}{1.3863} \times 10^3 \quad (4)$$

Thus mean taxonomic extinction rates can be calculated and expressed with the same unit for comparison between different fossil groups. Table 2 lists the calculated extinction rates for Neogene planktonic foraminiferal assemblages associated with their mean longevity. Apparently, the temperate assemblage has a shorter mean longevity and a higher extinction rate than that of tropical and transitional assemblages. However, the differences in mean longevity and extinction rates among these three climatic assemblages are not statistically significant. To test the prediction¹⁷ that 'the average evolutionary rate of planktonic foraminifera does not vary greatly with temperature' further, we have also grouped the total 94 extinct species into a more general warm assemblage (tropical plus warm subtropical) and a more general cool assemblage (temperate plus cool subtropical), and calculated the mean longevity and extinction rates (Table 2). The result indicates that the mean longevity and extinction rates are similar for both climatic groupings.

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A cytoplasmically transmitted disease of *Ceratocystis ulmi*

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The massive epidemics of Dutch elm disease occurring across much of the Northern Hemisphere from North America to Europe and south-west Asia are due to the spread of two highly pathogenic forms of *Ceratocystis ulmi*: the Eurasian (EAN) and the North American (NAN) races of the aggressive strain of the fungus¹⁻³. Most of the mature field elm population in these areas is likely to be destroyed as a result, and unless the pathogenicity of the EAN and NAN attenuates, successive generations of young elms can be expected to suffer a similar fate. Until now the factors that might contribute to any attenuation have remained a matter of speculation³, but I report here the discovery of a disease in the aggressive strain of *C. ulmi*, transmitted between mycelia by hyphal fusion, which severely reduces the growth and reproductive fitness of the fungus, and may be particularly severe when transmitted between NAN and EAN race isolates. It has features in common with transmissible hypovirulence in *Endothia parasitica*, the cause of the remission of chestnut blight in Europe. Its possible influence on the present Dutch elm disease epidemics and its potential as a disease control method are discussed.

The phenomenon was first noted when isolates of *C. ulmi* (NAN aggressive strain) were being paired in culture for tests on their vegetative (or somatic) compatibility. One isolate, H321, consistently caused the development of unusual areas of sunken and abnormal growth in 'recipient' cultures with which it was paired (Fig. 1a,b). When a sexually compatible recipient isolate was repeatedly back-crossed to H321 until the differences in their vegetative compatibility genes were removed, the effect was still present, showing that its origin did not lie in some aspect of vegetative incompatibility. This suggested that it was due to invasion of the recipient isolate's hyphae by H321 cellular inclusions.

To test this hypothesis subcultures were taken from the normal and abnormal regions of the recipient culture and paired against a normal 'self' as a recipient. The subcultures from the abnormal areas behaved as transformants, indicating that they had become infected by a transmissible factor. To test whether the transforming factor was cytoplasmic or nuclear, the nuclei of H321 were marked for tolerance to the fungicide MBC (a stable nuclear marker)⁴ and the fungicide-tolerant H321 was paired with a wild-type recipient. Subcultures taken from the abnormal and normal areas of growth developing in the recipient were then tested for fungicide tolerance, and for ability to transform a healthy culture (see Fig. 1d). None of the subcultures from the abnormal areas of mycelium was fungicide-tolerant, but they had all acquired the transforming factor. The factor is evidently a cytoplasmic determinant which can be transmitted between mycelia in the absence of nuclear exchange. I have termed it the disease or 'd'-factor.

Several d-infected isolates have since been obtained directly from nature, and the transmission of their d-factors to healthy recipient isolates investigated in paired cultures. Transmission is usually characterized by the development of sectors of weak or altered growth in the young recipient culture where it contacts the d-infected culture (Fig. 1c). These then either develop as expanding sectors of sunken infected mycelium, often giving a 'butterfly' appearance (Fig. 1d) or growth of the newly infected sector and subsequently that of much of the leading edge of the recipient culture ceases completely (Fig. 2a,b). The infected area is sometimes associated with the production of brown pigment (Fig. 2c); lines of pigment may also develop at

the boundary of infected and healthy areas of the recipient culture (Fig. 2d). Successive subcultures taken from the edges of developing recipient colonies indicate that the d-factor probably spreads in the recipient via fusion of growing hyphal tips. The patterns of infection thus vary according to the relative growth rates of the paired isolates, and of the healthy and newly d-infected areas of the recipient isolate. In the recipient, the d-factor can sometimes, but not always, be detected in subcultures taken from apparently normal mycelium adjacent to a visibly infected area (Fig. 1d).

Mycelial subcultures taken from d-infected sectors of a recipient usually show a much slower and sparser growth than their healthy parent on agar (Fig. 3). Initially some scarcely grow at all apart from a few hyphal strands (Fig. 3c) and later develop into an extremely slow-growing unstable culture type (Fig. 4). As this culture type is very similar to an 'amoeboid' degenerate type described previously for wild NAN aggressive isolates from nature⁵, pairing experiments were carried out using naturally occurring amoeboid isolates. They have not only confirmed that amoeboid isolates are indeed d-infected, but have also shown that they have a particularly severe effect when paired with a recipient (Fig. 2b).

In spore germination tests, 100 conidia were sampled from 6–12-day-old cultures of each of nine d-infected isolates; they showed a mean loss of viability of 42.4% (range 13–70%), and produced generally smaller and more irregular germ tubes than conidia from healthy counterparts. A variable proportion of the surviving colonies was detectably d-infected, while others

were apparently healthy. In contrast, when four d-infected isolates were sexually mated in two crosses and 15 F₁ progeny isolated from three perithecia of each cross, all 90 resulting progeny were apparently healthy. This indicates that the d-factor may be transmitted to the conidia but not to the sexual spores (ascospores) of the fungus.

To test whether transmission of a d-factor between mycelia is affected by vegetative incompatibility, d-infected isolates were paired with healthy isolates of the same or different vegetative compatibility (VC) groups. In one such experiment, a d-infected isolate was paired against 22 isolates of the same VC group (all VC genes the same) and 62 isolates of different VC groups (most VC genes different). Transmission of the d-factor occurred in all pairings with isolates of the same VC group, but in only six of the pairings with isolates from different VC groups. In another test a d-factor was transmitted more successfully to back-cross isolates having a majority of VC genes in common with the donor than to those with different genes at most VC loci. Vegetative incompatibility is therefore likely to impede d-factor transfer between mycelia.

From the above characteristics I conclude that the d-factor is a cytoplasmically transmitted disease of *C. ulmi*. By analogy with other transmissible diseases of fungi, the cytoplasmic factor could be associated with the mitochondria, as with senescence in *Podospora anserina*^{6,7} or *Aspergillus amstelodami*⁸, or could be associated with double-stranded (ds) RNA, possibly a mycovirus^{9,10}, as with degenerative diseases of *Helminthosporium victoriae*¹¹ and *Rhizoctonia solani*¹². The apparent non-transmission of the d-factor to the ascospores suggests that it is more likely to be dsRNA-associated. Certainly the d-factor shares many culture properties and transmission characteristics with the dsRNA-associated hypovirulence phenomenon or h-factor in the chestnut blight fungus *E. parasitica*^{13,14}, responsible for the unexpected remission of chestnut blight epidemics in Italy in the early 1950s, and now under investigation as a biological control agent in France and the USA^{14,15}. This raises the question of whether d-factors could exert a similar effect on Dutch elm disease, and how d-factor spread occurs in nature.

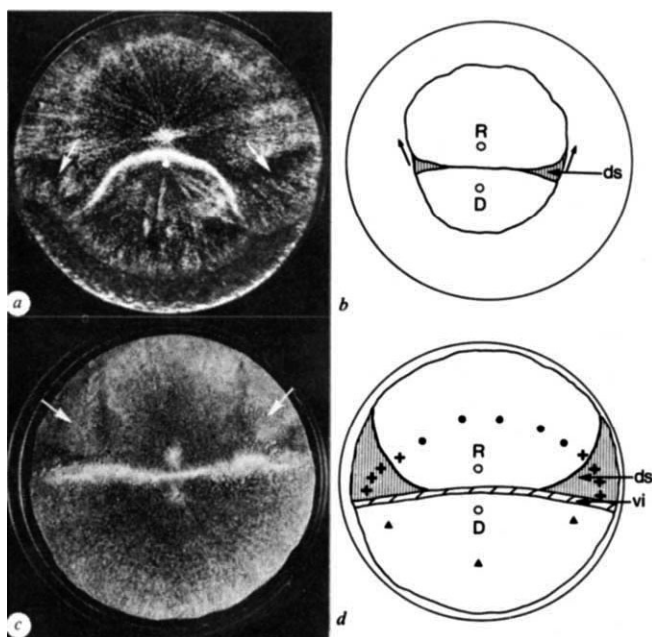


Fig. 1 a, Developing reaction between a slower growing d-infected donor isolate (below) and a healthy recipient isolate (above), showing dark sectors of abnormal newly infected growth in the recipient (arrowed). b, A developed reaction showing sectors of altered growth on each side of the recipient culture (arrowed). The white barrage between the two isolates in each case is the vegetative incompatibility reaction. Cultures were grown on elm sapwood agar¹⁸ at 20°C in darkness. c, Schematic representation of the initial reaction (that is, at a much earlier stage than a) between a d-infected donor isolate and a healthy recipient. Arrows indicate direction of d-factor spread in the recipient via fusion of peripheral d-infected and healthy hyphae. d, Schematic representation of a developed d-reaction. D, d-infected donor isolate; R, healthy recipient isolate; ds, newly d-infected colony sector; vi, vegetative incompatibility barrage. If the donor d-infected isolate carries a nuclear gene marker for fungicide tolerance, and after development of the d-reaction subcultures are taken from the points shown in b, the following phenotypes are obtained: ▲, fungicide-tolerant, transforming (d-infected); +, fungicide-sensitive, transformed; ●, fungicide-sensitive, healthy (or occasionally fungicide-sensitive, transformed).

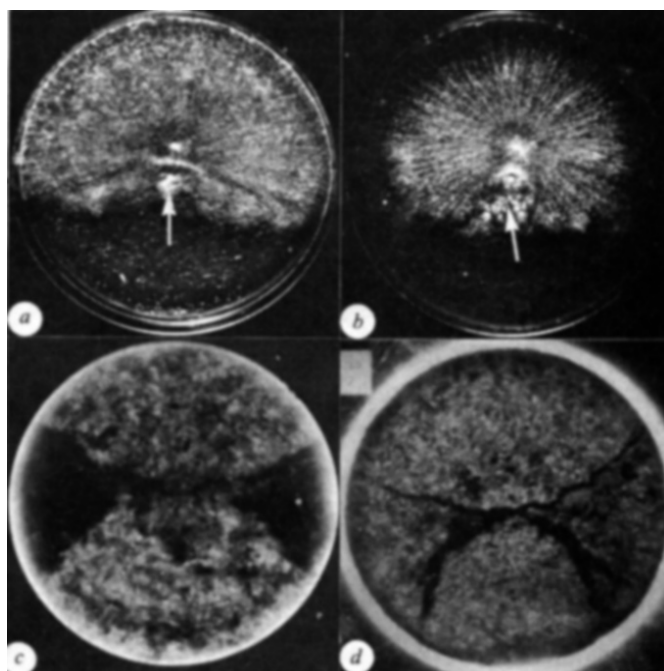


Fig. 2 a, b, Growth of a recipient culture: a, partially arrested; and b, completely arrested as a result of infection from a severely d-infected donor culture (very small colony, arrowed). c, Pigment development in the newly d-infected sectors of a recipient. d, Lines of pigment at the boundary of newly d-infected sectors in a recipient. c, d, Photographed with transmitted light. All cultures were grown on elm sapwood agar¹⁸ at 20°C.

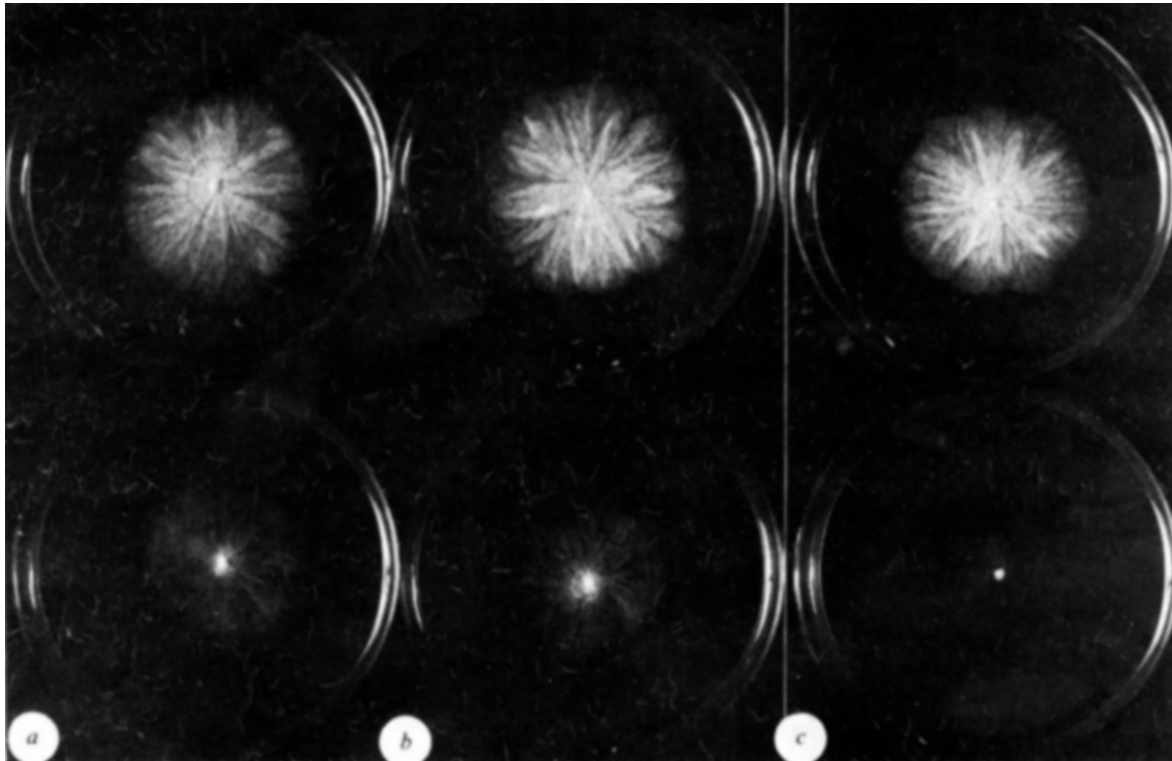


Fig. 3 Comparative growth of three healthy colonies (above) and their d-infected counterparts (below). *a, b*, NAN race aggressive strain isolates (H351 and H625); *c*, EAN race aggressive strain isolate H277 infected with a d-factor from a NAN isolate, showing the particularly severe effect of the resulting d-infection on growth. Cultures were grown on 2% Oxoid malt extract agar¹⁸ for 7 days at 20 °C.

The greatest opportunity for the spread of d-factors between mycelia must be during the long saprophytic phase of the fungus associated with beetle breeding galleries in the bark of diseased elm. An experiment in which healthy genetically marked isolates of *C. ulmi* were introduced into diseased bark has confirmed that the marked isolates can acquire d-factor infection from other isolates in the bark; other samples of bark have sometimes revealed discrete zones of 'amoeboid' naturally d-infected mycelium (Fig. 4). At the end of the bark phase, d-infected spores will be carried away by newly emerging beetles, and further opportunity for transfer of d-factors may occur in the feeding grooves cut by the beetles in twig crotches of healthy elms (the point at which infection of healthy elms by *C. ulmi* occurs). When the beetles re-enter bark of dying elms to breed they will again introduce d-infected spores which will colonize the breeding galleries.

The potential influence of d-factors on the current Dutch elm disease epidemics has to be viewed in the context of major changes occurring in the composition of the *C. ulmi* population. The EAN and NAN races of the aggressive strain responsible for the present epidemics are moving rapidly into areas once occupied only by the 'old' non-aggressive strain responsible for the epidemics of the 1920s³. An important question is whether the two races of the aggressive strain will survive through the post-epidemic period and continue to attack the next generation of young elm suckers and seedlings, or whether they will be replaced again by the non-aggressive strain (or even by aggressive × non-aggressive hybrids)³. Recent evidence suggests that only the two forms of the aggressive strain will survive, and that the non-aggressive strain and any hybrids will be virtually eliminated³. In this case the future of the elm looks bleak unless the pathogenicity of the aggressive strain attenuates in some way. As pathogenicity in *C. ulmi* is substantially under the control of nuclear genes^{16,17}, some attenuation might occur through mutation and sexual recombination.

The present work, however, raises the further possibility that attenuation might be brought about by d-factor or a similar

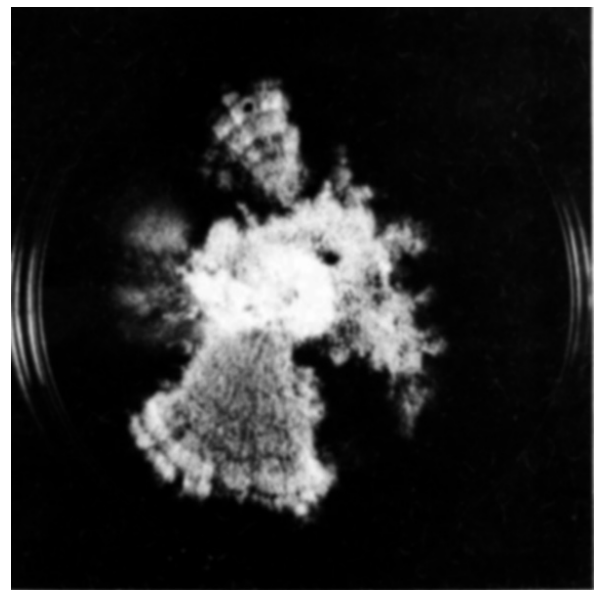


Fig. 4 An unstable 'amoeboid' colony type resulting from severe d-infection; 2-week-old culture on 2% Oxoid malt extract agar¹⁸.

infection causing a critical reduction of fitness in the aggressive strain population, similar to that which has occurred with hypovirulence in chestnut blight. Indeed, the impact of the d-factors on *C. ulmi* is likely to be greater in the aftermath of the epidemics when the *C. ulmi* population is small and potentially more responsive to selection imposed by a reduced host and vector population than during the epidemics. Loss of fitness could result from reduction in growth vigour and sporulation in the saprophytic (bark) phase, from a consequent reduction in the quantity and viability of spore inoculum on emerging beetles, and also from any possible direct effect, so

far undetermined, on pathogenicity. Furthermore, as the three strains/races of *C. ulmi* are genetically widely separated^{2,17}, they may carry qualitatively different d-factors. An effect of their recent intermixing in the present epidemics could be the transfer of d-factors between them, possibly with particularly deleterious consequences for recipient mycelia (Fig. 3c). Additional loss of fitness in the aggressive strain population, therefore, might be brought about by infection with d-factors from the non-aggressive strain or from the sister race of the aggressive strain.

The comparison with hypovirulence in chestnut blight also raises the possibility, albeit remote, that d-factors might be used in the control of Dutch elm disease, for example by releasing vector beetles carrying d-infected *C. ulmi* in order to

infect healthy *C. ulmi* in beetle breeding galleries. The present results, however, indicate that both artificially promoted and natural spread of d-factors in nature could be impeded by the fungus' vegetative incompatibility system. Other limiting factors could be the rate of transfer of d-factors through sexual and asexual spores and through the pathogenic yeast-like phase in host xylem, d-factor dosage effects and qualitative differences between d-factors. These aspects, together with the molecular nature of d-factors and their interaction with the nuclear genome, including the pathogenicity genes, are under investigation.

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Multi-oscillatory control of circadian rhythms in human performance

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Circadian rhythms are known to exist in many measures of human performance efficiency as well as in physiological processes¹. The demands of a task, and in particular its 'working memory'² load, play a large part in determining the time of day at which it is best performed³. Furthermore, task demands may affect the speed with which performance rhythms adjust to the altered sleep/wake schedules occasioned by shift-work and rapid time-zone transitions⁴. These differences in rate of adjustment may be explained by a similar multi-oscillatory model to those proposed for physiological rhythms^{5–10}. These assume any given circadian rhythm to be jointly controlled by two endogenous oscillators. The first is thought to be relatively immune to exogenous factors and to control the temperature rhythm, while the second is thought to be more influenced by exogenous factors and to have the major role in governing the sleep/wake cycle. Normally, the pronounced 24-h time cues, or 'zeitgebers', in our environment result in both oscillators, and hence all circadian rhythms, running with a period of 24 h. However, under altered sleep/wake schedules, and in conditions of temporal isolation, the temperature rhythm and sleep/wake cycle may separate from one another and run with distinctly different periods. When such 'internal desynchronization' occurs, other physiological rhythms have been found to run in synchrony with one or other of these two functions. This finding forms the basis of current multi-oscillatory models. However, studies of abnormal sleep/wake schedules suggest that the rhythm in working memory performance may sometimes separate from both the sleep/wake cycle and temperature rhythm by running with a period of less than 24 h¹¹. We have investigated this possibility here and our results indicate control of working memory performance rhythms by a previously unidentified oscillator with an autonomous period of about 21 h.

Our studies used Wever's 'fractional desynchronization' technique¹². This technique induces internal desynchronization of different overt rhythms by the progressive shortening or lengthening of successive 'days' by means of artificial zeitgebers. Seven volunteer subjects were individually isolated from all known natural zeitgebers for about 28 days in an isolation unit⁶. They were exposed to the artificial zeitgebers of a light/dark cycle of the available lights, and regularly occurring gong signals. The gong signals occurred six times during each light period (at 3-h intervals on a 24-h time scale) and once during each dark period (equidistant between the adjacent 'light' gong signals). Three of the subjects were run on a shortening schedule in which the period of these zeitgebers was progressively shortened by 5 min per cycle from 24.0 to 22.0 h. The other four subjects were run on a lengthening schedule in which the zeitgebers were progressively lengthened by 10 min per cycle from 26.0 to 29.0 h. Core body temperature was monitored continuously by means of a rectal probe, while sleep onset and offset times were estimated from button pushes made by the subjects on retiring and on awakening. In all subjects, the sleep/wake cycle followed the changes in the zeitgebers' period for the duration of the study.

The gong signals cued the subjects to perform standard paper and pencil versions of a simple letter cancellation test and of a more complex verbal reasoning test. The former involved searching through lines of 30 random capital letters (arranged in 3 blocks of 15 lines each) line by line, cancelling all the 'E's. The latter consisted of 32 sentences (for example, 'A is not preceded by B'), and the subject's task was to indicate whether each sentence was a 'true' or 'false' description of the letter pair following it (that is, AB or BA)¹³. In normal conditions, performance speed on this test reaches a maximum at about mid-day¹⁴, somewhat earlier than on the letter cancellation test, but later than on more memory-loaded tasks with a higher 'working', memory component³. Two of the subjects also performed low (1 target), medium (3 target) and high (5 target) memory-loaded versions of a search and memory (SAM) test. This is an improved version of MAST¹⁵, and each version involved searching through 18 lines of 60 single-spaced pseudo-random capital letters searching for the occurrence of any one of the 1, 3 or 5 letters in the 'target set' defined at the beginning of each line. The time taken to complete each of these tests was recorded automatically in the control room of the research unit.

The resultant performance data were smoothed by means of a 3-point moving average (weighted 1-2-1) and then 24-h

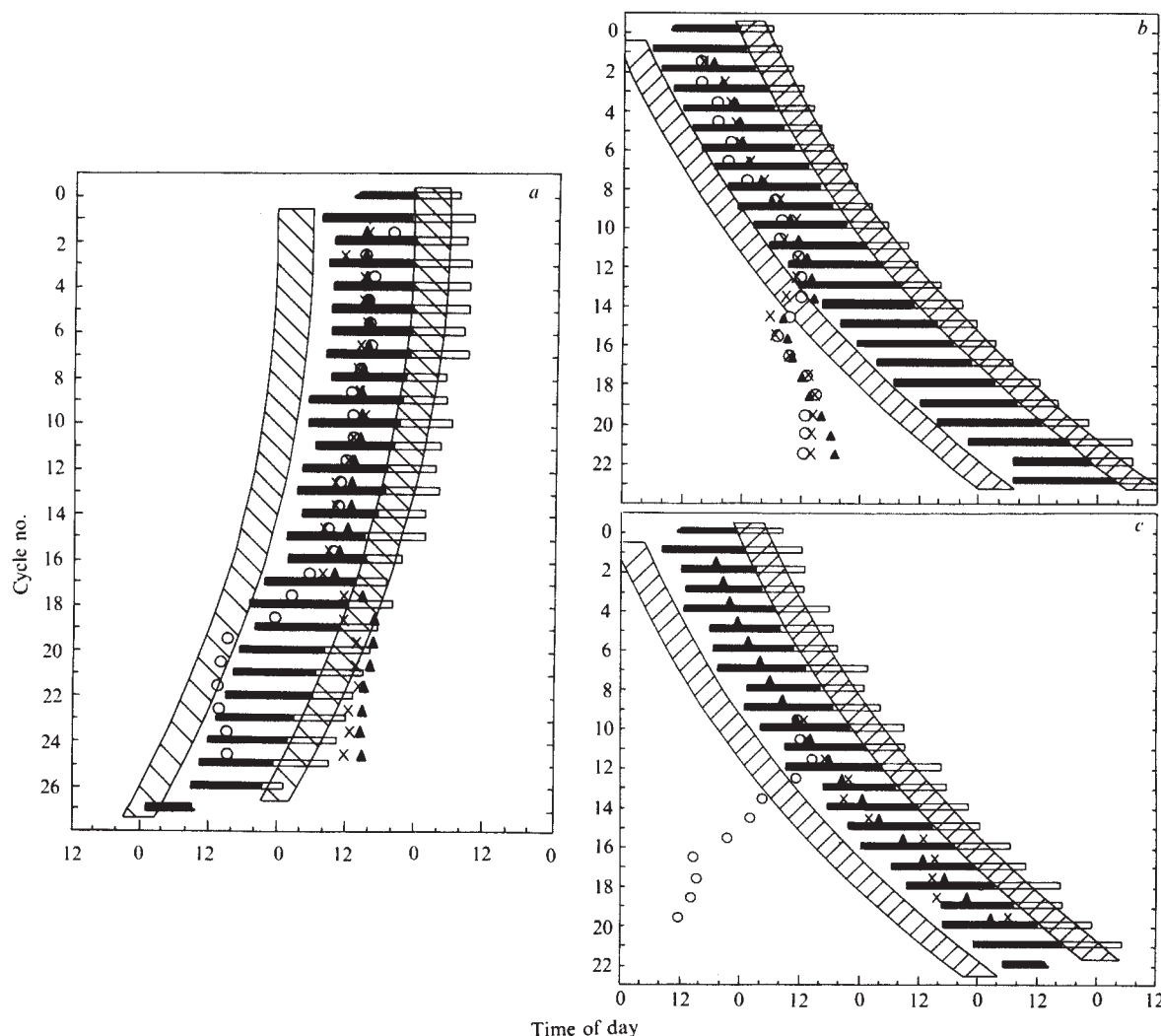


Fig. 1 The estimated peak (acrophase) of the circadian rhythms in letter cancellation (x) and logical reasoning (O) performance, and in body temperature (▲), on successive light/dark cycles. The shaded areas represent the dark period, the solid bars the periods of wakefulness, and the open bars the periods of sleep. *a* Shows the results from a subject (267) run on a shortening schedule, and *b* and *c* the results from two subjects (269 and 272) run on a lengthening schedule.

sinusoids were fitted to successive overlapping 3-cycle windows to estimate the 'peak' (or acrophase)^{16,17}. A similar analysis was performed on the temperature data to ensure comparability of the results, although this resulted in slightly different estimates from those reported elsewhere¹². Following their separation from the sleep/wake cycle at their 'limit of entrainment', the free-running period of the various rhythms was estimated by means of least-squares linear regression analysis of the acrophase values.

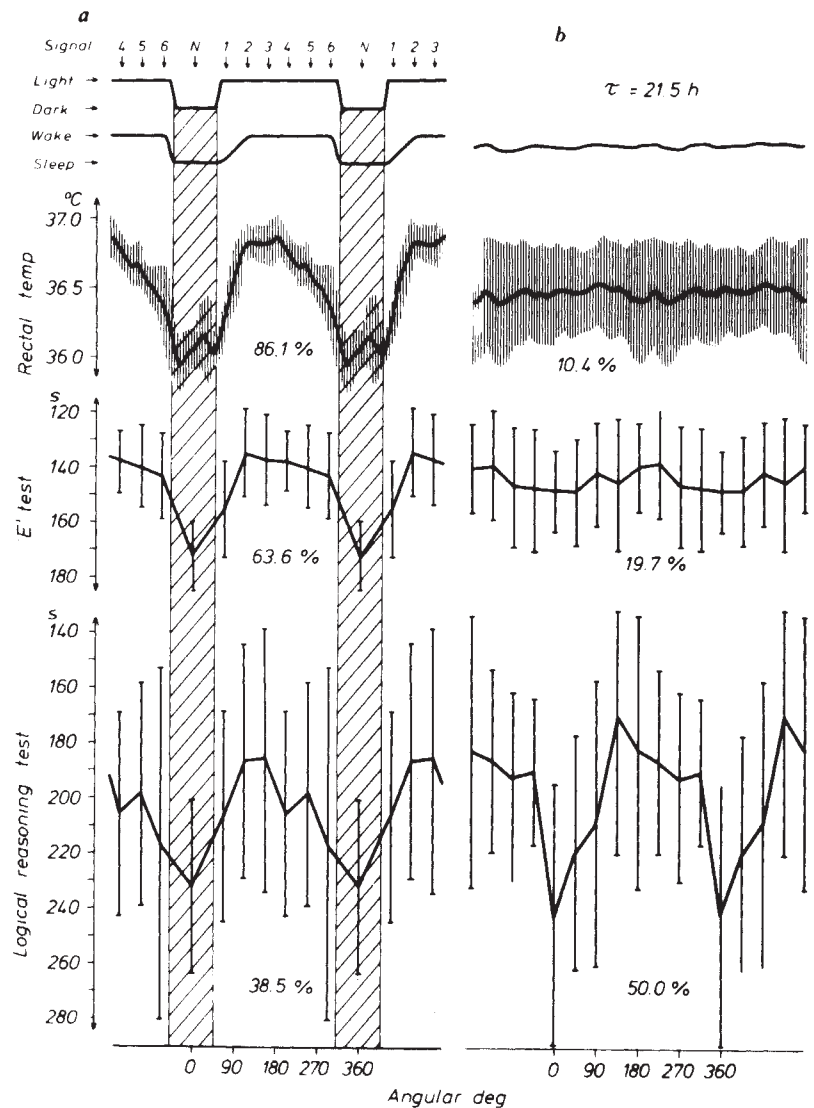
The findings for temperature, letter cancellation and verbal reasoning are summarized in Table 1, together with details of the subjects' age and sex. In all the subjects, the rhythm in letter cancellation performance very closely followed that in body temperature (see Fig. 1). In contrast, three subjects showed evidence of a separation of their verbal reasoning rhythm from these other two rhythms at some stage during the study (for example, Fig. 1*a, c*). In all three cases, the rhythm in verbal reasoning achieved this separation by running with a

Table 1 Limit of entrainment and subsequent free-running period of the circadian rhythms in temperature, 'E' cancellation and verbal reasoning performance

Subject	Temperature		'E' cancellation		Verbal reasoning	
	Limit of entrainment	τ free-run	Limit of entrainment	τ free-run	Limit of entrainment	τ free-run
'Shortening studies'						
259 (♂19 yr)	22.8	24.3	22.7	24.5	22.7	24.4
260 (♀21 yr)	23.1	25.2	23.2	25.3	22.9	25.2
263 (♀21 yr)	22.8	24.4	22.8	24.4	22.9	20.5/24.2
'Lengthening studies'						
261 (♂17 yr)	26.8	24.5	26.5	24.5	26.0	21.2/24.4
264 (♂35 yr)	26.7	24.4	26.5	24.3	26.7	24.3
269 (♂35 yr)	27.5	25.1	27.3	24.9	27.3	24.5
272 (♂23 yr)	>29.0	NA	>29.0	NA	27.5	21.5

NA, data not available.

Fig. 2 The data from subject 272 'educated' on the zeitgeber period (a) and on a 21.5-h period (b). The percentages below each plot indicate the power of the rhythms⁶.



period of about 21 h. In two cases (261 and 263) it subsequently became re-synchronized with the temperature rhythm (Table 1), but in the third case (272) it ran with a period of 21.5 h for the last 13.5 objective days of the study (Fig. 1c).

Although this latter subject performed the tests throughout the 28 days, the data for the first eight cycles are unavailable due to an apparatus failure. This subject also differs from the others in that he was subjected to a high level of artificial illumination (~4,000 lx) during the light periods which resulted in his temperature rhythm remaining entrained for the duration of the study¹⁸. His 'educated rhythms', or time of 'day' functions for the last 13.5 objective days of the study are shown in Fig. 2. Figure 2a shows the functions calculated for a zeitgeber 'day', while Fig. 2b shows the 21.5-h 'day' functions. Verbal reasoning performance showed clear evidence of a 21.5-h rhythm ($P < 10^{-5}$), but this was not reflected in either the letter cancellation or temperature data. Further, the fit to the zeitgeber's period found for verbal reasoning may, at least in part, be due to a 'masking' effect of the sleep/wake cycle. Such an effect may also have increased the standard deviations of the readings when cast on a 21.5-h period, but cannot have contributed to the fit obtained at this period.

These results suggest that, at least in some subjects, verbal reasoning performance is controlled by an underlying oscillator with an autonomous period of about 21 h. One explanation for the failure to find a 21-h rhythm in all subjects is that, due to differences in mental ability, this task may tax some subjects' working memory system more than others. A 21-h rhythm may only become evident when this system is taxed by the task

demands. Evidence to support this suggestion was obtained from the SAM test performed by two of the subjects. In both subjects, performance on the low memory-load (one-target) version of this test followed body temperature quite closely, while that on the high memory-load (five-target) version separated from body temperature and ran with a period of about 21 h (see Fig. 3). As was the case with the verbal reasoning task, the results from the intermediate memory-load (three-target) version of this SAM test suggest only partial control by a 21-h oscillator.

In conclusion, these findings suggest that simple, non memory-loaded performance is controlled by the oscillator responsible for body temperature, while more taxing, memory-loaded, performance is controlled by a 21-h oscillator. In addition, there is evidence that the oscillator responsible for the sleep/wake cycle exerts an influence over circadian performance rhythms^{6,19}. We would thus view performance rhythms as under the control of a similar multi-oscillatory system to that proposed for physiological rhythms, but with the addition of a previously unidentified oscillator with an autonomous period of about 21 h. It is unclear which other variables are controlled by this latter oscillator, although the fractional desynchronization technique used here may afford a method for identifying these in future studies.

Finally, these findings may have important practical implications in shiftwork and rapid time-zone transition situations where people are commonly required to perform mentally taxing, memory-loaded tasks.

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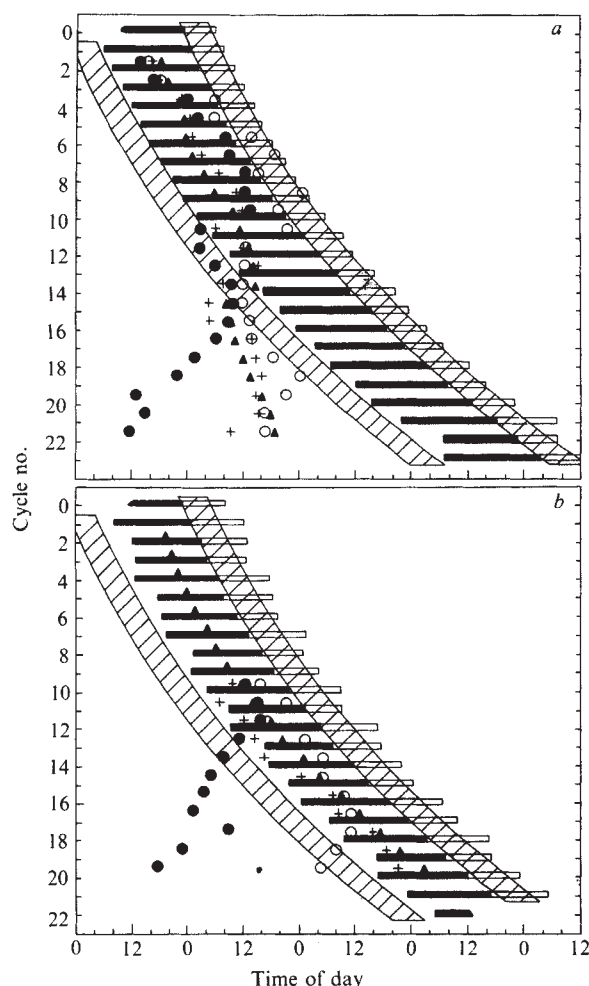


Fig. 3 The estimated peak (acrophase) of the circadian rhythms in one-target (+), three-target (○) and five-target (●) SAM performance, and in body temperature (▲), on successive light/dark cycles. The shaded areas represent the dark period, the solid bars the periods of wakefulness, and the open bars the periods of sleep. *a* Shows the results from subject 269, and *b* the results from subject 272.

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Added noise restores recognizability of coarse quantized images

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When a portrait is coarsely quantized into blocks, the block structure hides the face, although lower spatial frequencies of the original image sufficient by themselves for recognition are preserved. Recognition can be recovered by blurring the image, or otherwise attenuating the spurious higher spatial frequency components^{1,2}. Harmon and Julesz² claim that high spatial frequencies introduced by quantized blocking mask the lower spatial frequencies which convey information about the face, preventing recognition. Here we show that recognition can be enhanced, without decreasing the amplitude of these spurious higher frequencies, by adding further high-frequency noise to the quantized image. This result is clearly at odds with a theory of high-frequency (or critical band) masking. We suggest that the added noise mutes mechanisms which would otherwise impose a block structure on the image, allowing the alternative perceptual organization of the hidden face to reemerge.

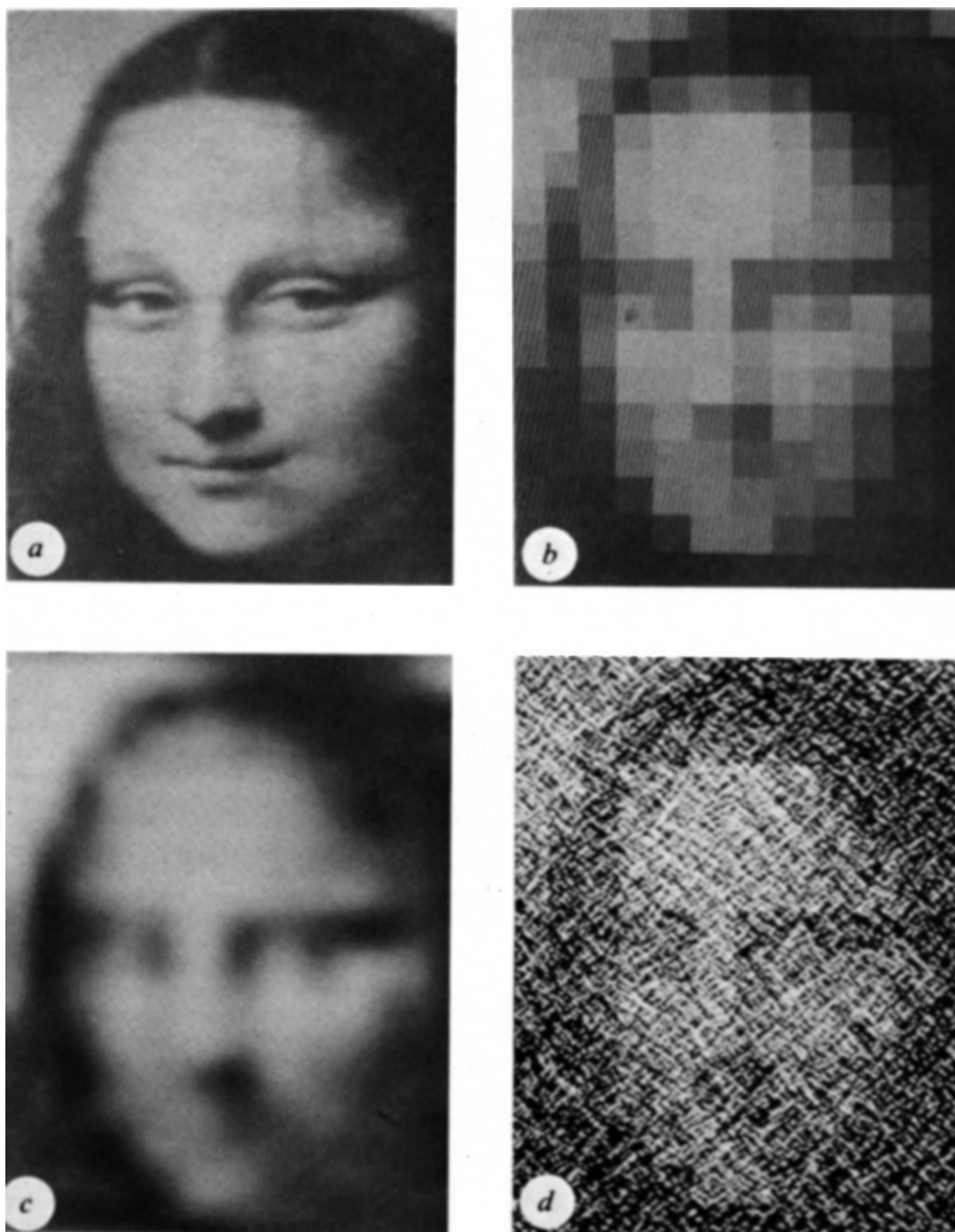
A detail of Leonardo's 'Mona Lisa' was digitized into 256 × 256 pixels, using a flying spot scanning system made up of a Cromemco microcomputer, an oscilloscope (Hewlett Packard 1335A), a lens and a photometer (Spectra Pritchard 1980A). The digitized file was transferred to a larger computer (PDP-11/44) which performed all the necessary mathematical manipulations. Processed files were transferred back to the Cromemco, which displayed the resultant images on a variable persistence oscilloscope (Hewlett Packard 1335A). Figure 1*a* shows an unmanipulated version of the picture to give an indication of the image quality of the system.

The 256 × 256 pixel picture was next reduced to 16 × 16 blocks, by setting all the elements in each block of 16 × 16 pixels to a uniform luminance equal to the mean luminance of the elements of that block (Fig. 1*b*). Technically, the effect of this quantization is to remove the high spatial frequencies of the original picture and replace them with spurious high spatial frequencies associated with the block structure of the new image. Lower frequencies sampled above the Nyquist rate are left relatively intact.

As Harmon and Julesz² report, recognition can be recovered by attenuating the spurious high spatial frequencies, either by screwing up the eyes, by defocusing, by reducing the image size, contrast or luminance (to a point where vision can no longer resolve the higher frequencies) or by filtering. Figure 1*c* shows a low pass version of the quantized image: the face looks blurred, but is more recognizable than the image from which it is derived (Fig. 1*b*).

The original explanation for the failure to perceive faces after coarse quantization was that spectrally adjacent higher frequencies introduced by the quantization process mask the lower frequencies which convey information about the face, an idea which is supported by experiments demonstrating critical band masking both in vision³ and in hearing⁴. Appealing though this notion is⁵, however, the idea of the high-frequency components masking the low-frequency components becomes less credible when their relative power is considered. Figure 2 shows that the spectrum of high spatial frequencies of the blocked portrait is meagre in comparison with that of the lows, both in amplitude and in spread of orientations. It is difficult to conceive how these high spatial frequency components are capable of corrupting the image beyond recognition.

Fig. 1 *a*, The face of Leonardo's 'Mona Lisa' after it had been digitized into 256×256 pixels (256 grey levels), Fourier transformed, back-transformed and photographed using a time exposure technique. *b*, Coarse quantized version of *a*, produced by setting all the elements within each block of 16×16 pixels to a uniform luminance equal to the mean luminance of the elements within that block. *c*, Low pass version of *b*, produced by removing all frequencies of period less than 1.5 times the block size. *d*, The blocked portrait with superimposed high-frequency noise (see Fig. 2).



We tested Harmon and Julesz's theory by adding further high-frequency noise to the quantized Mona Lisa. This noise comprised only high spatial frequencies (higher than one and a half times the block frequency) and had energy everywhere except within 22.5° of the horizontal and vertical meridians, where the energy of the high-frequency components of the quantized image lies. Thus, it increased the power and spread of the high frequencies without physically interfering with the edges of the block structure, or reducing amplitude by phase cancellation. If the masking theory² were correct, this barrage of added noise could be expected only to contaminate the picture further.

Figure 1*d* shows the result. Rather than impeding recognition, added high-frequency noise significantly enhances it. Although the edges are still visible on scrutiny, the noise destroys the block structure of the quantized picture, allowing Leonardo's Gioconda to reemerge. Several observers have claimed that the quantized picture with added noise is of a higher quality than

the low pass filtered version (Fig. 1*c*), rather like a pointillist version of the original.

This curious result suggests that the effect of the noise is to destroy the configuration introduced by blocking. One possible mechanism emerges on considering the physiological effect of visual noise. Simple cells in cat cortex do not respond to two-dimensional visual noise⁶. Burr *et al.*^{7,8} have claimed that the silence of simple cells results from inhibition by surrounding cortical cells of different orientation preference, which is supported by the fact that simple cells respond well to one-dimensional visual noise which is mathematically contained within the two-dimensional noise to which they fail to respond. Simple cells prefer isolated one-dimensional stimuli—lines, edges, gratings or noise. This preference for one-dimensional stimuli led Burr *et al.*^{7,8} to suggest that simple cells may be specialized to signal the contours or boundaries of objects, which are locally one-dimensional. It is conceivable that in human vision there may also exist mechanisms similar to simple cells in cat cortex which

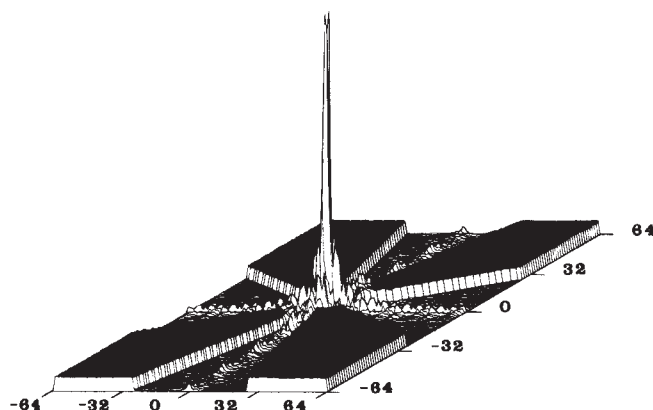


Fig. 2 Spectral representation of the image in Fig. 1*d* with mean luminance set to zero. Spatial frequency is indicated on the abscissae of the base plane (in cycles per picture height) and amplitude is represented along the ordinate. The high peaks in the centre are the low-frequency components of the original image. The ripples along the meridians are the spurious high-frequency components introduced by coarse quantization (giving rise to the block structure of Fig. 1*b*). The elevated and shaded 'cross' formation is the transform of the added high-frequency orientation band-limited noise. This noise contains no low-frequency energy, and none within 22.5° of the horizontal and vertical meridians, but is flat elsewhere.

are similarly susceptible to inhibitory control, and which may form part of a more general set of structuring mechanisms, serving to disentangle objects from their background.

In the block portrait of Fig. 1*b*, the clearest one-dimensional structures are the outlines of the blocks themselves. Structuring mechanisms of appropriate spatial frequency and orientation tuning (analogous to simple cells) will form the sketchlines of the framework within which the portrait is perceived. Adding noise of all orientations except that of the blocks will generate inhibition of the output of the structuring mechanisms tuned to high frequencies, weakening the block organization and allowing the alternative 'Mona Lisa' organization to take over. She too has contours which, for short runs at least, are more or less one-dimensional. These contours should excite structuring mechanisms of lower spatial frequency tuning (uninhibited by the high-frequency noise), which dictate the alternative perceptual organization of the portrait (which was presumably previously overridden by the high-frequency response).

As the noise in Fig. 1*d* contains no energy within 22.5° of that of the spurious high frequencies (Fig. 2), it should not mask these frequencies (in the sense that it raises their threshold), as classical masking is small⁹ at 22.5° (although 22.5° may be within the range of complicated phenomena such as disadaptation¹⁰). Thus, we are not suggesting that the added noise makes the spurious high-frequency components of the blocked figure invisible and thereby unmasks the low-frequency components. Rather, we assert that the added noise destroys the propensity to organize the image according to its spurious high-frequency structure, by inhibiting the mechanisms which create that organization.

This is not to say that the particular noise used in Fig. 1*d* is the only stimulus which will restore recognition. White random noise and various grid patterns will all do the job, to a greater or lesser extent. So also will jumbling the phase of the spurious components introduced by blocking. It is possible that in these conditions, other mechanisms, such as masking, may play a part in restoration of recognition.

Other types of explanation than those in terms of inhibition and masking also deserve more consideration than we have yet given them. For example, we have not speculated about interactions between energy in different spatial frequency bands, nor about the effect on the alignment of zero crossings¹¹ of added noise and other stimuli. Further work along these lines is in progress.

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Quantification of Ca^{2+} -activated K^+ channels under hormonal control in pig pancreas acinar cells

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Ca^{2+} - and voltage-activated K^+ channels are found in many electrically excitable cells and have an important role in regulating electrical activity^{1–4}. Recently, the large K^+ channel has been found in the baso-lateral plasma membranes of salivary gland acinar cells, where it may be important in the regulation of salt transport⁵. Using patch-clamp methods^{6,7} to record single-channel currents from excised fragments of baso-lateral acinar cell membranes in combination with current recordings from isolated single acinar cells and two- and three-cell clusters, we have now for the first time characterized the K^+ channels quantitatively. In pig pancreatic acini there are 25–60 K^+ channels per cell with a maximal single channel conductance of about 200 pS. We have quantified the relationship between internal ionized Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) membrane potential and open-state probability (p) of the K^+ channel. By comparing curves obtained from excised patches relating membrane potential to p , at different levels of $[\text{Ca}^{2+}]_i$, with similar curves obtained from intact cells, $[\text{Ca}^{2+}]_i$ in resting acinar cells was found to be between 10^{-8} and 10^{-7} M. In microelectrode experiments acetylcholine (ACh), gastrin-cholecystokinin (CCK) as well as bombesin peptides evoked Ca^{2+} -dependent opening of the K^+ conductance pathway, resulting in membrane hyperpolarization. The large K^+ channel, which is under strict dual control by internal Ca^{2+} and voltage, may provide a crucial link between hormone-evoked increase in internal Ca^{2+} concentration and the resulting NaCl-rich fluid secretion^{5,8}.

Figure 1*A* shows a series of single-channel current recordings from an excised patch in quasi-physiological conditions: that is, the extracellular solution was in contact with the outside of the patch membrane (pipette solution) and intracellular solution was on the inside of the plasma membrane (bath solution). When the ionized Ca^{2+} concentration in the bath ($[\text{Ca}^{2+}]_i$) was kept at 10^{-8} M there were only few and brief unitary outward currents at negative membrane potentials. Depolarizing the membrane patch increased the frequency of channel opening events. At membrane potentials of 30–40 mV, the one channel present in the patch was almost constantly open except for very brief moments of closure. These results are very similar to those obtained when recording from electrically isolated patches *in situ* (not shown). The single-channel current-voltage (i/V) relationship is shown in Fig. 1*B* (circles). Reversal of outward to inward current was not observed, but it is clear that the null potential is more negative than –40 mV. In this case the only ion with a negative equilibrium potential is K^+ ($E_K = -82$ mV), as there is no Cl^- gradient.

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Elevating $[Ca^{2+}]_i$ to 10^{-7} M increased the proportion of time the channel spent in the open state; this effect was particularly marked at negative membrane potentials (Fig. 1A). At extreme depolarizations the channel sometimes went from the open state into a state of prolonged closure (top right panel in Fig. 1A). The single-channel i/V relationship at $[Ca^{2+}]_i = 10^{-7}$ M was identical to that obtained at $[Ca^{2+}]_i = 10^{-8}$ M (Fig. 1B). Three experiments of the type shown in Fig. 1A were carried out, giving similar results.

Unitary activity could also be observed in excised patches exposed to symmetrical K^+ solutions (five experiments), but not

with symmetrical Na^+ solutions (three experiments). A typical linear single-channel i/V relationship in a K^+/K^+ situation is shown in Fig. 1B (triangles), representing a single-channel conductance of about 200 pS (see also Fig. 2). The i/V relationship was not dependent on $[Ca^{2+}]_i$ (Fig. 1B), but the open-state probability, at negative membrane potentials, increased markedly after increasing $[Ca^{2+}]_i$ to 10^{-7} M.

Figure 1C shows the relationship between membrane potential and the open-state probability of the K^+ channel in the presence of quasi-physiological Na^+ and K^+ concentration gradients. Three curves obtained from experiments on excised

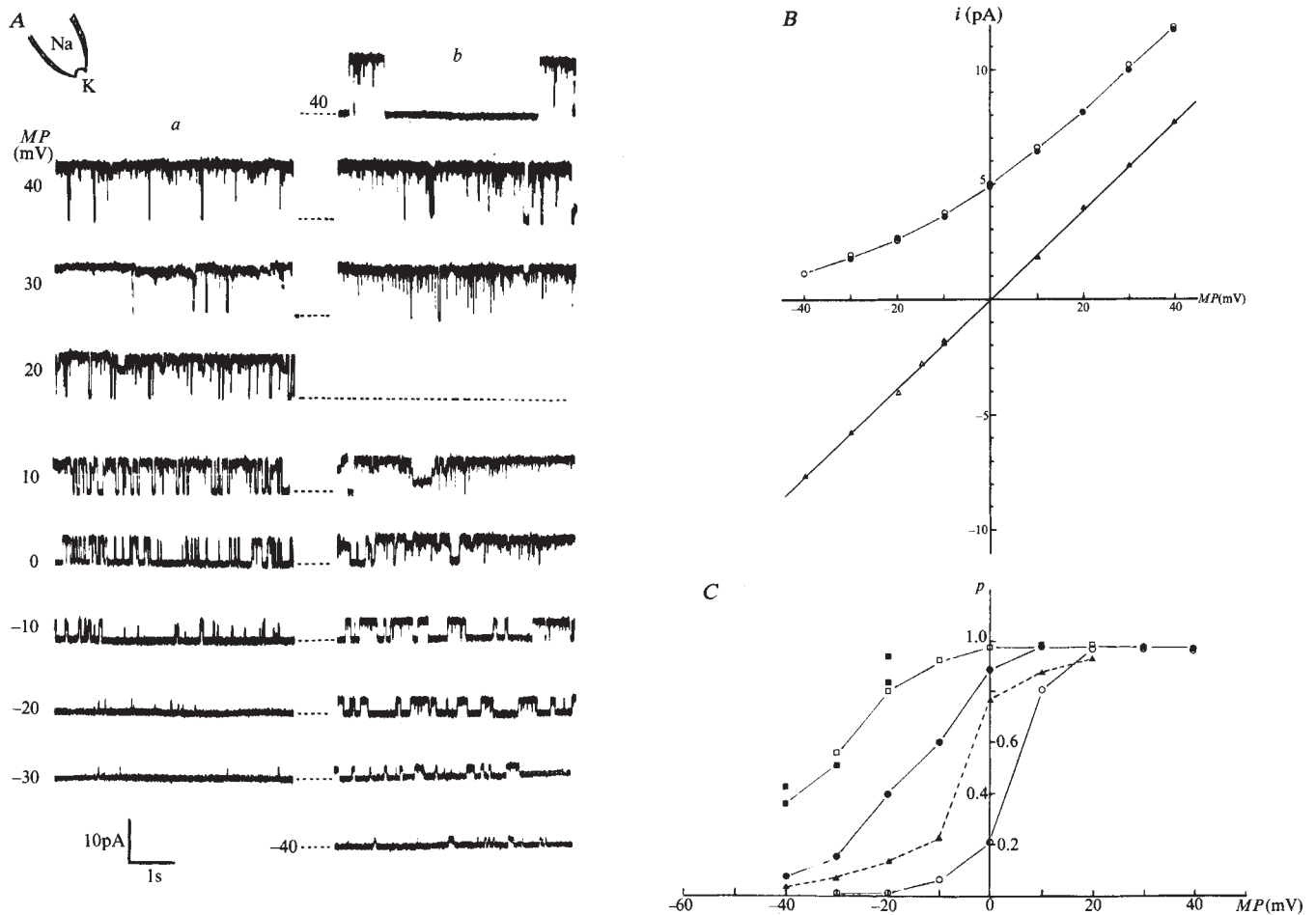


Fig. 1 Single-channel current recording from excised (inside-out) pig pancreatic acinar membrane patches. **A**, Single-channel current traces from an inside-out membrane patch exposed to extracellular solution on the outside (pipette) and intracellular solution on the inside (bath). The extracellular solution contained (mM): 140 NaCl, 4.7 KCl, 1.2 $CaCl_2$, 1.13 $MgCl_2$, 10 glucose, 10 HEPES, pH 7.2. The intracellular solution had the following composition (mM): 145 KCl, 10 NaCl, 1.13 $MgCl_2$, 10 glucose, 10 HEPES, pH 7.2. In the part of the experiment represented in **a** the ionized Ca^{2+} concentration in the bath ($[Ca^{2+}]_i$) was 10^{-8} M (0.2 mM Ca^{2+} and 2.2 mM EGTA). At the time when the traces shown in **b** were obtained from the same patch, $[Ca^{2+}]_i$ was adjusted to 10^{-7} M (1 mM Ca^{2+} and 2 mM EGTA). The membrane potentials (MP) at which the individual traces were obtained are indicated. The current level when the channel is closed is indicated by horizontal dashed lines. Upward deflections represent outward current. Two traces represent the 10^{-7} M situation at a membrane potential of 40 mV. This is to indicate that while the channel was generally open there were times when the channel went from the open state into a state of prolonged closure. All the traces are only short, but are typical excerpts of much longer recordings. **B**, Single-channel current-voltage (i/V) relationships obtained from experiments on excised membrane patches. The circles represent the experiment shown in **A** ('normal' Na^+/K^+ gradients). The closed circles represent $[Ca^{2+}]_i = 10^{-8}$ M while the open circles represent $[Ca^{2+}]_i = 10^{-7}$ M. The triangles are from a different experiment in which the intracellular solution was used on both sides of the membrane (K^+/K^+ situation). The pipette solution contained 1.2 mM $CaCl_2$ while $[Ca^{2+}]_i = 10^{-8}$ M in the measurements represented by the closed triangles and $[Ca^{2+}]_i = 10^{-7}$ M in the situation represented by the open triangles. **C**, Curves showing the relationship between open-state probability of K^+ channel and membrane potential at three different levels of $[Ca^{2+}]_i$: open circles 10^{-8} M, closed circles 10^{-7} M and open and closed boxes 10^{-6} M. All the experiments were carried out in the presence of normal Na^+/K^+ gradients. The open and closed circles represent the experiment shown in **A**. The open-state probability was determined by examining 30–40-s long continuous stretches of recordings at each potential level. The triangles (dashed line) represent results from an experiment on an *in situ* patch. The pipette and bath solution was the usual extracellular solution. The potential across the patch membrane was calculated as the difference between the normal cell membrane potential (–60 mV) (see Fig. 2) and the pipette potential. $[Ca^{2+}]_i$ in the intact cell was apparently between 10^{-7} M and 10^{-8} M.

Methods: Pig pancreata were obtained at the local abattoir. Small fragments (50 mg) were injected with physiological saline containing pure collagenase (Worthington) (100 U/ml) and incubated with the same type of collagenase solution at 37 °C for 30 min (once or twice). At the end of this period there were large undigested lumps, clusters containing from a few up to 20–30 acinar cells as well as some isolated single acinar cells. The cells or cell clusters were rinsed with collagenase-free physiological saline solution for 15 min to 1 h before use. The technique for single-channel recording and excision of membrane patches was as described by Neher and collaborators^{6,7}.

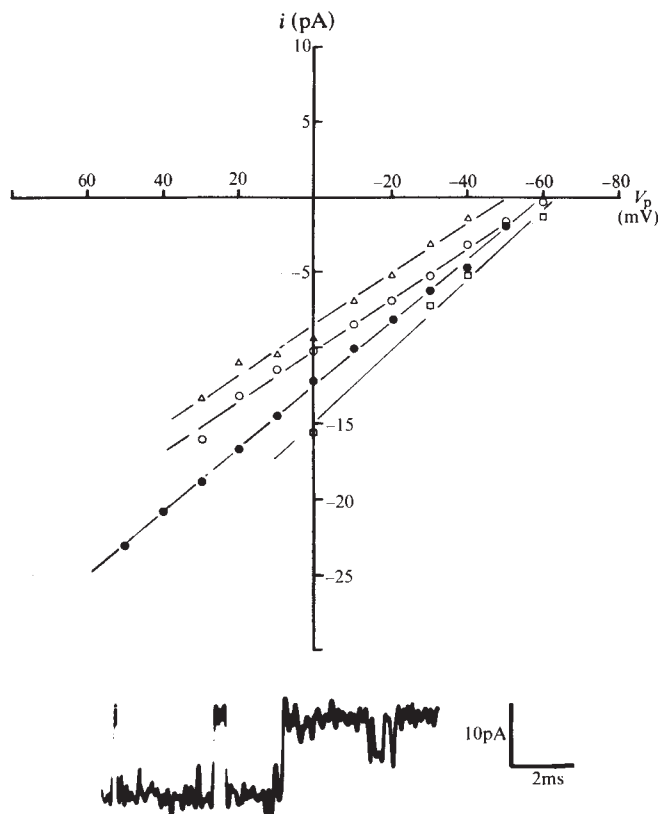


Fig. 2 Plots of single-channel current as a function of pipette potential from patches *in situ* (cell attached conformation) with intracellular solution in the pipette and extracellular solution in the bath. Closed circles represent an experiment carried out with Cl-free sulphate solutions whereas the open symbols represent three other experiments in which solutions with Cl⁻ rather than sulphate were used. The null potential in all cases is close to $V_p = -60$ mV. As there is no K⁺ gradient across the patch, this indicates that the membrane potential of these cells is about -60 mV. Inset shows a high-time resolution trace of single-channel current from an *in situ* patch with intracellular solution in the pipette. The pipette potential was 0 mV. In addition to the full openings and closures in the left part there is an incomplete opening (or opening of smaller channel) in the right part. In K⁺/K⁺ situations such smaller current steps were frequently encountered. Only the large steps were used in the plots of i/V_p relations.

patches are shown relating to three different internal (bath fluid) Ca²⁺ concentrations. The curves are shifted to the left by about 20–30 mV for a 10-fold increase in [Ca²⁺]_i. Figure 1C also shows the relationship between open-state probability of the K⁺ channel and membrane potential in an intact cell based on single-channel recordings from an electrically isolated patch *in situ*. The relationships between the open-state probability curves *in situ* and in the excised patches (Fig. 1C) indicate that [Ca²⁺]_i in the intact cell is below 10⁻⁷ M but above 10⁻⁸ M.

Figure 2 shows plots of single-channel current as a function of pipette potential, from membrane patches *in situ* (cell attached conformation), exposed to high K⁺ solution in the pipette, but with normal extracellular solution in the bath. As the pipette was filled with intracellular solution there is no K⁺ concentration gradient across the membrane patch. The resting potential should therefore be equal to the pipette potential at which the single-channel currents reversed polarity or attained a zero value since

$$i = g(V_m - V_p)$$

where i is the single-channel current, g the single-channel con-

ductance, V_m the membrane potential and V_p the pipette potential. The i/V_p relationship is linear in all cases and the intercept with the V_p axis occurs at about -60 mV.

Figure 3A shows the currents flowing across the plasma membranes of a single cell, two connected acinar cells and a three-cell cluster in response to hyperpolarizing or depolarizing voltage steps. Depolarizing voltage steps resulted in large outward currents whereas hyperpolarizing steps were associated with only very small inward currents. Figure 3B shows the steady-state relationship between current and voltage (closed circles, α) obtained from the two-cell experiment represented in Fig. 3A(b). This total current (I) must be composed of a leakage current and the current through the K⁺ channels (I_K). The leakage current is assumed to be linear and is obtained by extrapolating the I/V relationships found for the potentials more negative than the holding potential (-60 mV) (γ). I_K can be accounted for by

$$I_K = Npi$$

where N is the number of K⁺ channels in the unit, p the open-state probability and i the single-channel current. For comparison, Fig. 3B also includes a plot of the i/V relation (β) taken from Fig. 1B (circles). The product of channel number and open-state probability (Np) can therefore simply be calculated from Fig. 3B by $Np = (\alpha - \gamma)/\beta$, where α is the total current (I), γ is the leakage current and β the single-channel current (i). Plots of Np as a function of membrane potential for the eight experiments carried out in one-, two- and three-cell situations are shown in Fig. 3C. From Fig. 1C it can be seen that the open-state probability is close to 1 at a membrane potential of $+20$ mV and this is confirmed by the results from the one-cell situations. As shown in Fig. 3C, the number of K⁺ channels per cell (resting state) ranges between 25 and 60 (mean value: 45 ± 11 (s.e.)).

Figure 4 shows examples of conventional intracellular micro-electrode recordings from isolated superfused tissue segments. The resting membrane potentials ranged widely from -15 to -65 mV, with a mean value of -31 mV ± 10 mV (s.d.) ($n = 64$). The most negative potentials correspond to the magnitude of the resting potential as estimated by the single-channel current null potential method (Fig. 2). The lower potentials no doubt reflect varying degrees of leaks around the inserted microelectrodes⁹. A number of agonists such as ACh, pentagastrin (CCK5) and bombesin nonapeptide, evoked hyperpolarization of the acinar membrane. There was a linear relationship between amplitude of stimulant-evoked hyperpolarization and magnitude of resting potential. Extrapolation indicated that the ACh or CCK5 reversal potential was about -80 to -90 mV, that is, very close to E_K . A 10-fold increase in external K⁺ concentration shifted E_{CCK5} to values between -20 and -30 mV, that is, a shift of about 60 mV, which is in agreement with the predicted shift in E_K (Fig. 4). The pancreatic secretagogues therefore act to increase the membrane K⁺ conductance. The stimulant-evoked increase in K⁺ conductance was dependent on the presence of external Ca²⁺. Sustained ACh stimulation (5×10^{-7} M) resulted in sustained hyperpolarization (minutes). In the absence of external Ca²⁺ (Ca²⁺-free, 10^{-4} M EGTA), the first sustained ACh stimulation produced only a transient hyperpolarization and the second period of stimulation did not result in any membrane potential change. Similar results were obtained in four experiments. The microelectrode results from the pig pancreatic acinar cells resemble more closely results from lacrimal and salivary glands (particularly cockroach salivary acini¹⁰) than the pattern observed in the mouse and rat pancreas where ACh, CCK and bombesin invariably evoke depolarization⁹ due to the high density of Ca²⁺-activated non-discriminatory cation channels¹¹.

In addition to the information obtained about the number of K⁺ channels per cell and the regulation of this channel by internal Ca²⁺ and membrane potential, our data also allow conclusions about [Ca²⁺]_i. The value in resting acinar cells of 10^{-8} – 10^{-7} M

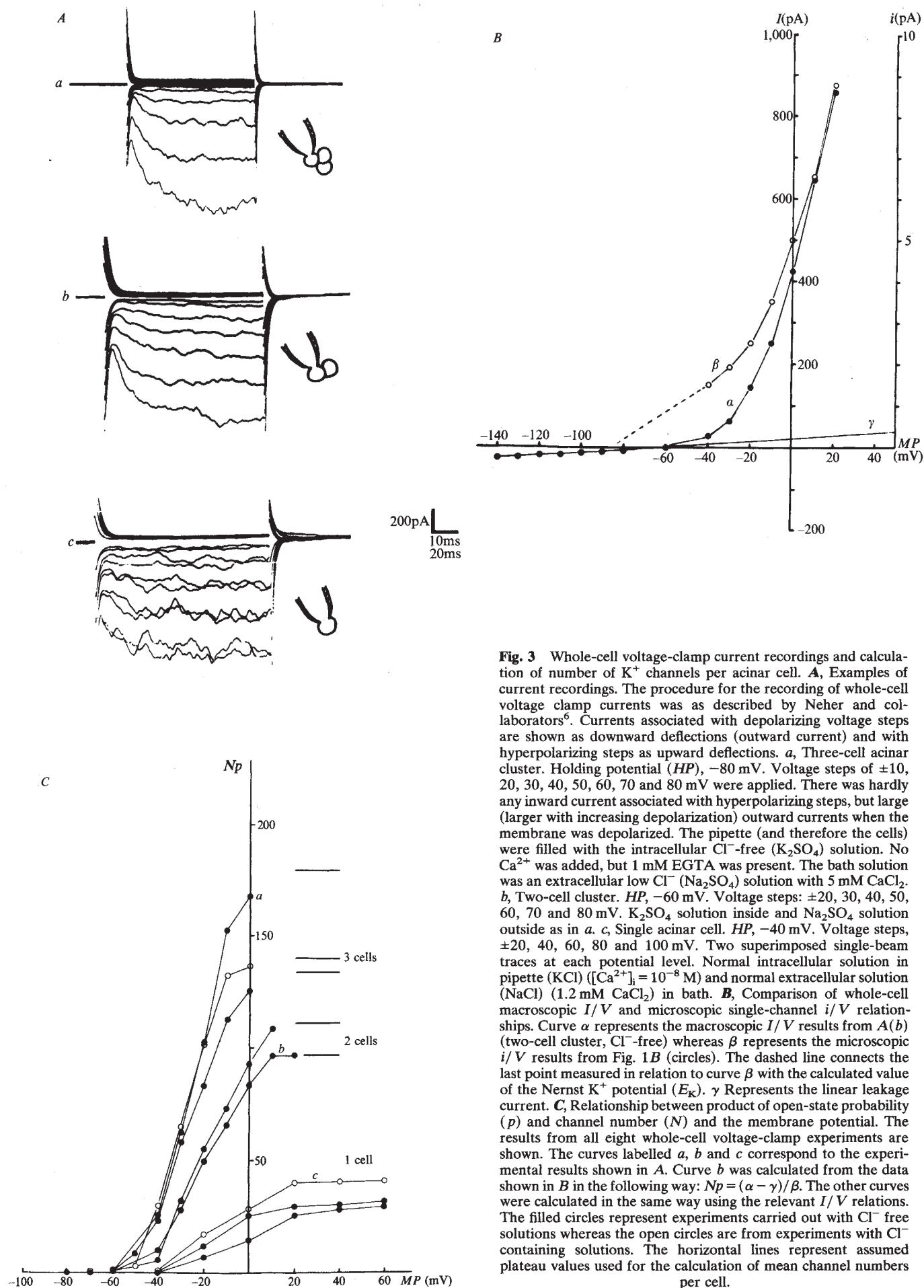


Fig. 3 Whole-cell voltage-clamp current recordings and calculation of number of K^+ channels per acinar cell. **A**, Examples of current recordings. The procedure for the recording of whole-cell voltage clamp currents was as described by Neher and collaborators⁶. Currents associated with depolarizing voltage steps are shown as downward deflections (outward current) and with hyperpolarizing steps as upward deflections. *a*, Three-cell acinar cluster. Holding potential (HP), -80 mV. Voltage steps of ± 10 , 20, 30, 40, 50, 60, 70 and 80 mV were applied. There was hardly any inward current associated with hyperpolarizing steps, but large (larger with increasing depolarization) outward currents when the membrane was depolarized. The pipette (and therefore the cells) were filled with the intracellular Cl^- -free (K_2SO_4) solution. No Ca^{2+} was added, but 1 mM EGTA was present. The bath solution was an extracellular low Cl^- (Na_2SO_4) solution with 5 mM $CaCl_2$. *b*, Two-cell cluster. HP, -60 mV. Voltage steps: ± 10 , 20, 30, 40, 50, 60, 70 and 80 mV. K_2SO_4 solution inside and Na_2SO_4 solution outside as in *a*. *c*, Single acinar cell. HP, -40 mV. Voltage steps, ± 20 , 40, 60, 80 and 100 mV. Two superimposed single-beam traces at each potential level. Normal intracellular solution in pipette (KCl) ($[Ca^{2+}]_i = 10^{-8}$ M) and normal extracellular solution (NaCl) (1.2 mM $CaCl_2$) in bath. **B**, Comparison of whole-cell macroscopic I/V and microscopic single-channel i/V relationships. Curve α represents the macroscopic I/V results from *A*(*b*) (two-cell cluster, Cl^- -free) whereas β represents the microscopic i/V results from Fig. 1B (circles). The dashed line connects the last point measured in relation to curve β with the calculated value of the Nernst K^+ potential (E_K). γ Represents the linear leakage current. **C**, Relationship between product of open-state probability (p) and channel number (N) and the membrane potential. The results from all eight whole-cell voltage-clamp experiments are shown. The curves labelled *a*, *b* and *c* correspond to the experimental results shown in *A*. Curve *b* was calculated from the data shown in *B* in the following way: $Np = (\alpha - \gamma)/\beta$. The other curves were calculated in the same way using the relevant I/V relations. The filled circles represent experiments carried out with Cl^- free solutions whereas the open circles are from experiments with Cl^- containing solutions. The horizontal lines represent assumed plateau values used for the calculation of mean channel numbers per cell.

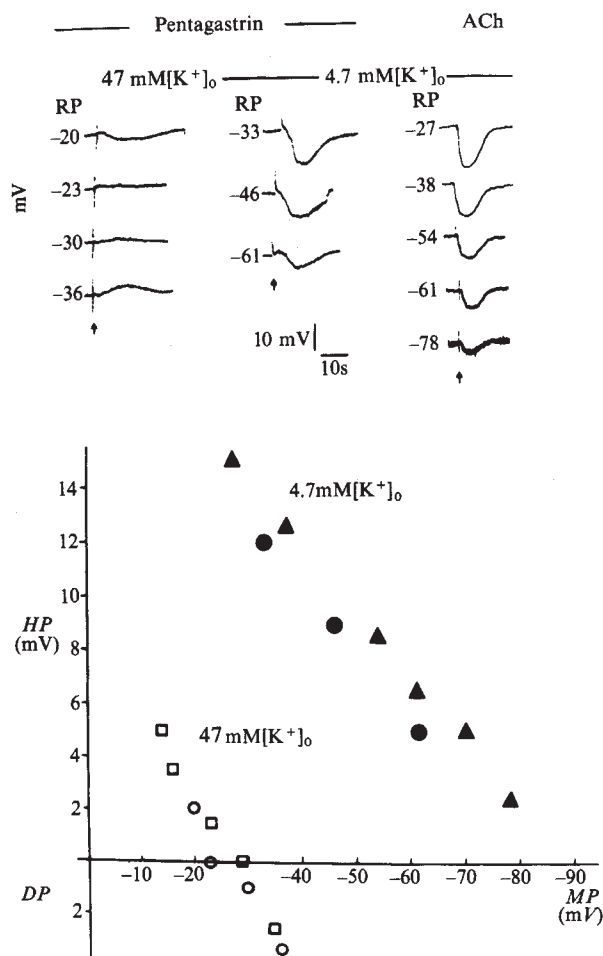


Fig. 4 The effects of transmitters and hormones on membrane potential in conventional microelectrode experiments on superfused pig pancreatic segments. Two intracellular microelectrodes inserted into communicating acinar cells were used. Direct current (hyper- or depolarizing) was applied through one of the electrodes while the other monitored the potential¹⁰. Stimulation by micro-iontophoresis of ACh (extracellular pipette filled with 2 M ACh-Cl, 60 nA ejection current for 100 ms, 20 nA retaining current) or of pentagastrin (CCK5) (3×10^{-4} M CCK5, 1,000 nA ejection current for 400 ms, no retaining current) in the presence of atropine (10^{-6} M). The amplitudes of the ACh- or CCK5-evoked potential changes are plotted as a function of the resting potential. When the bathing fluid K⁺ concentration was elevated to 47 mM the Na⁺ concentration was reduced correspondingly to ensure constant osmolality. □, ○, ●, CCK5; ▲, ACh.

(Fig. 1C) is lower than that previously obtained from studies with Ca²⁺-sensitive microelectrodes¹². In microelectrode studies there are, however, frequently serious leak problems and in the previous study¹² the resting potentials were indeed very low. As we have quantified the K⁺ channel and its control mechanisms, we can in principle calculate the magnitude of the acinar membrane hyperpolarization in response to a given increase in [Ca²⁺]_i. The microelectrode responses to ACh or CCK5 stimulation shown in Fig. 4 could be accounted for by assuming that [Ca²⁺]_i had increased from about 5×10^{-8} M to about 1 μ M.

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Corticotropin-releasing factor is a potent inhibitor of sexual receptivity in the female rat

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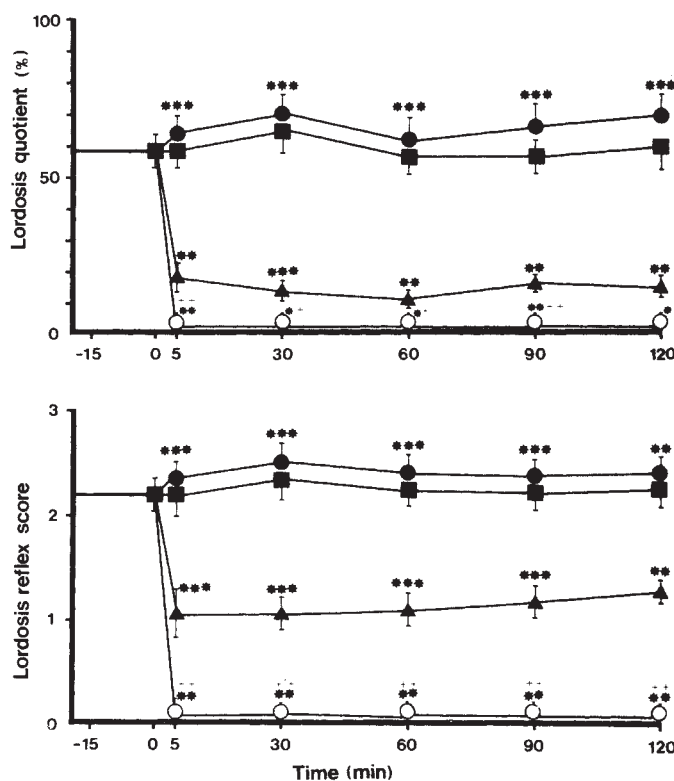
Corticotropin-releasing factor (CRF), the recently characterized and synthesized 41-amino acid polypeptide^{1,2} isolated from ovine hypothalamus, has been shown to be a potent stimulator of adenohypophyseal β -endorphin and corticotropin (ACTH) secretion both *in vitro*^{2–6} and *in vivo*^{2,4,7,8}. In common with other regulatory peptides, CRF has also been demonstrated to possess extra-hypophysiotropic roles. Indeed, intracerebroventricularly (i.c.v.) administered CRF elicits several endocrine and behavioural responses compatible with the concept that this peptide could be a key signal in coordinating the organism's endocrine and behavioural responses to stressful and other adaptive stimuli^{8–14}. We now provide the first evidence for neurally placed CRF in the control of a specific hormone-dependent behavioural response and unequivocally demonstrate an extremely potent suppressive effect of CRF on sexual behaviour in the female rat when microinfused into the arcuate-ventromedial area of the hypothalamus (ARC-VMH) and the mesencephalic central grey (MCG).

The rationale for investigating the action of CRF on female sexual receptivity is based on the observation that β -endorphin is a potent inhibitor of lordosis behaviour in the female rat¹⁵. We assumed that there could be a close neuroanatomical relationship between the CRF and β -endorphin/ACTH neuronal systems and that if CRF could stimulate the secretion of β -endorphin and ACTH from the pituitary gland, it could also do so in central nervous system (CNS) sites containing β -endorphin/ACTH neuronal networks; thus, some of the effects of CRF could be ascribable to the β -endorphin and ACTH released in response to CRF. The effects of CRF could then be abolished by immunoneutralization of the released β -endorphin or ACTH by pretreatment of the CNS site with a specific antiserum to either β -endorphin or ACTH. This assumption of an intimate neuroanatomical relationship between opioid and CRF neuronal systems seemed feasible in view of the recent demonstration of a coexistence of dynorphin(1–8) and CRF peptides in a subpopulation of neurones in the paraventricular nucleus of the hypothalamus (PVN)¹⁶.

The two CNS sites chosen to assess the effects of CRF on lordosis behaviour in the female rat, the arcuate-ventromedial area of the hypothalamus^{17,18} and the MCG^{15,19,20} are both intimately involved in the neural circuitry for lordosis, the former also containing β -endorphin/ACTH-immunoreactive perikarya^{21,22} and the latter β -endorphin/ACTH-immunoreactive fibres²². Recent immunocytochemical and immunological studies have shown a wide distribution of CRF-like

Fig. 1 The effect of saline (■), CRF (▲), anti- β -endorphin antiserum+CRF (●) and anti-ACTH antiserum+CRF (○) infusions into the arcuate-ventromedial area of the hypothalamus on lordosis behaviour in oestrogen-primed female rats assessed in terms of the lordosis quotient and the lordosis reflex score. Each time point denotes the mean $\pm$ s.e.m. of 11 rats. Asterisks (** $P < 0.01$, *** $P < 0.001$, Student's *t*-test) denote statistically significant differences between groups (CRF versus saline; anti- β -endorphin antiserum+CRF versus saline; CRF versus anti- β -endorphin antiserum+CRF. Crosses (+ $P < 0.05$, ++ $P < 0.01$) denote statistically significant differences between CRF versus anti- β -endorphin antiserum+CRF.

Methods: Stainless steel 29-gauge cannulae were chronically implanted bilaterally into the ARC-VMH and MCG in ovariectomized Wistar female rats (200–220 g) under sodium pentobarbital anaesthesia (50 mg per kg body weight) using a Kopf stereotaxic instrument fitted with atraumatic ear bars. The stereotaxic coordinates of Pelligrino *et al.*³⁵ were used for the ARC-VMH and MCG but using the modifications of Wishaw *et al.*³⁶ to maintain the angle formed between the upper incisor bar and the interaural line at $8^{\circ}29'$ for each rat. To demonstrate the potent suppressive effects of CRF on female sexual behaviour, animals with moderately high levels of lordosis were used. To achieve consistent adequate levels of lordosis in all the animals, 2 days after cannulation each female was injected subcutaneously with 5 μ g oestradiol benzoate in 0.1 ml peanut oil and then with 50 ng oestradiol benzoate for 3 consecutive days. They were tested on the last day of this treatment (day 4) for sexual receptivity by pairing with a proven sexually vigorous male. CRF was prepared in saline at a concentration of 1 μ g μ l⁻¹. Samples (10 μ l) of undiluted rabbit anti-human β -endorphin antiserum were lyophilized and stored at -20°C . They were resuspended in 5 μ l saline at the time of the experiment. The binding capacity of the antiserum was 3 μ g ml⁻¹. Antibody formation is directed to the C-terminal end of the β -lipotropin (β -LPH) molecule. Specificity studies showed that anti- β -endorphin antiserum cross-reacts with β -LPH in equimolar proportions. However, the antiserum showed no cross-reactivity ($<0.01\%$) with [Met]enkephalin, [Leu]enkephalin, LHRH, somatostatin, CRF or substance P. Samples (10 μ l) of undiluted rabbit anti-porcine ACTH(1–36) antiserum were lyophilized and stored at -20°C . They were resuspended in 10 μ l of saline at the time of the experiment. The binding capacity of the antiserum was 10 μ g ml⁻¹. It showed 50% cross-reactivity with ACTH(1–24) but no cross-reactivity ($<0.1\%$) with α -MSH, β -LPH, β -endorphin, α -endorphin, LHRH, somatostatin or CRF. The antisera were each infused 15 min before CRF. Each solution was prepared immediately before use and infused in a volume of 2 μ l over 2 min using a Harvard infusion pump (Model 975). Dye-marker studies showed that this procedure produced no significant diffusion of the solution outside the desired brain site. All the infusions were done with 31-gauge stainless steel tubes fitted into the guide cannulae and protruding 0.5 mm from the tips of the latter. Two sets of inner cannulae and Hamilton microsyringes (10 μ l) were used for the simultaneous delivery of each solution into the bilateral guide cannulae. The internal infusion cannulae were left in place for 1 min after the end of the infusion before withdrawal. During this infusion procedure the animal was held in a restrainer but not anaesthetized. The female was then paired with a sexually vigorous male and lordosis reflex score (LS) and the lordosis quotient (LQ) (number of lordoses $\times$ 100/number of mounts) were assessed. The lordosis reflex score was determined using the scale 0 (no vertebral dorsiflexion) to 3 (full dorsiflexion). The LS and LQ were assessed immediately after the end of the infusion and then every 30 min. The LQ was calculated as per cent lordoses per 20 mounts. The average of 20 ratings of the reflex score was collected independently by two investigators. At the end of the experiment the animals were killed and the brains removed for histological confirmation of the placement of cannulae tips.



immunoreactivity throughout the CNS with CRF-like immunoreactive cell bodies located mainly in the PVN^{23,26}. However, cell bodies were detected in other areas of the brain including the arcuate nucleus²⁴, the MCG²³ and CRF-like immunoreactive fibres in the arcuate nucleus^{25,26} and VMH^{23,26}, and the midbrain^{23,25,26} including the MCG^{23,26}, thus indicating a possible interaction between CRF and β -endorphin/ACTH neurones.

The infusion of 2 μ g CRF (0.3 nmol) into the ARC-VMH induced a prompt significant inhibition of the lordosis response in the oestrogen-primed female rat (Fig. 1). This level of inhibition lasted for ~ 5 h. A smaller dose of CRF (0.15 nmol) did not significantly inhibit lordosis, and CRF (0.3 nmol) infused outside the ARC-VMH, for example in the ventral premammillary nucleus, had no effect on lordosis. Pretreatment of the ARC-VMH site with specific anti- β -endorphin antiserum completely prevented the CRF inhibition of lordosis behaviour (Fig. 1), demonstrating that the suppressive effects of CRF may be mediated via β -endorphin. Pretreatment of the ARC-VMH site with rabbit anti-human serum albumin antiserum did not prevent the CRF inhibition of lordosis behaviour. However, even with larger doses of CRF (3 nmol), complete abolition of the lordosis response could not be obtained, as was found with β -endorphin infused alone into the MCG¹⁵. We therefore assumed that ACTH released together with β -endorphin in

response to CRF could account for this because i.c.v. infusions of ACTH can facilitate female sexual behaviour^{27,28} and in our study anti-ACTH antiserum alone infused into the ARC-VMH significantly depressed lordosis behaviour in the oestrogen-primed female (data not shown). There is agreement that β -endorphin, α -melanotropin (α -MSH) and ACTH are derived from the same precursor molecule, proopiomelanocortin^{29,30} and all three peptides are found in the same neurones in the arcuate nucleus^{21,31}. Thus, both β -endorphin and ACTH could be released from the same neurone in response to the same stimulus (CRF) to regulate lordosis behaviour. Therefore, immunoneutralization of the released ACTH with the specific anti-ACTH antiserum could allow the full expression of the suppressive effect of the β -endorphin released in response to CRF. The results clearly show that when the ARC-VMH site was pretreated with anti-ACTH antiserum, there was a prompt total abolition of the lordosis response with 0.3 nmol CRF (Fig. 1). Rabbit anti-human serum albumin antiserum had no effect.

This profound inhibition of lordosis behaviour produced by CRF was accompanied by aggressive behaviour on the part of each female tested, with active rejection of the male, for example, kicking, rolling over and fending off the male during mounting attempts. There was a three-fold increase in the number of rejections when anti-ACTH antiserum was used with CRF.

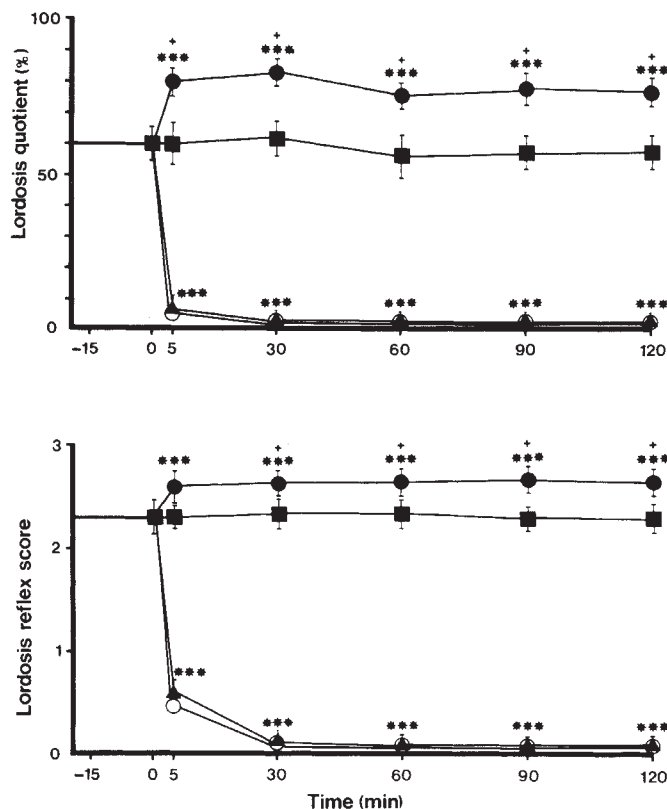


Fig. 2 The effect of saline (Δ), CRF ($\blacksquare$), anti- β -endorphin antiserum+CRF ($\bullet$) and anti-ACTH antiserum+CRF ($\circ$) infusions into the mesencephalic central grey on lordosis behaviour in oestrogen-primed female rats. Each time point denotes the mean $\pm$ s.e.m. of 10 rats. Asterisks *** ($P < 0.001$ Student's *t*-test) denote statistically significant differences between groups (CRF versus saline; anti-ACTH antiserum+CRF versus saline; CRF versus anti- β -endorphin antiserum+CRF). Crosses + ($P < 0.05$) indicate significant differences between anti- β -endorphin antiserum+CRF versus saline.

The infusion of CRF (0.3 nmol) into the MCG completely abolished lordosis behaviour in oestrogen-primed female rats within 30 min (Fig. 2). This inhibition lasted for about 5 h. Pretreatment of the MCG site with anti- β -endorphin antiserum not only overcame the CRF-induced suppression of lordosis behaviour but promptly potentiated lordosis above control values even though these were already high (Fig. 2). Pretreatment of the MCG site with anti-ACTH antiserum caused no further suppressive effects on lordosis by CRF but there was a fourfold increase in the number of female rejections. Anti-ACTH antiserum alone infused into this brain site significantly inhibited lordosis behaviour (data not shown); however, any facilitatory effect of ACTH released in response to CRF was not evident in the MCG. Although β -endorphin and ACTH are stored in the same neurone it is unclear whether the same terminal releases both peptides or whether preferential release of one peptide occurs at different sites in different physiological conditions or in response to a given stimulus. The complete suppression of lordosis by CRF in the MCG and the total reversal of this blockade by anti- β -endorphin antiserum may indicate that β -endorphin is preferentially released in the MCG in these conditions to affect lordosis. CRF had no effect when infused into the superior colliculus, which contains CRF²⁶ but no β -endorphin fibres²¹, and pretreatment of the MCG site with anti-human serum albumin antiserum did not prevent the CRF-induced abolition of lordosis behaviour.

β -endorphin in the MCG inhibits lordosis in the female rat by inhibiting the release of luteinizing hormone releasing hormone (LHRH)¹⁵; moreover, anti- β -endorphin antiserum alone infused into the MCG promptly potentiates lordosis in oestrogen-primed female rats by enhancing LHRH release

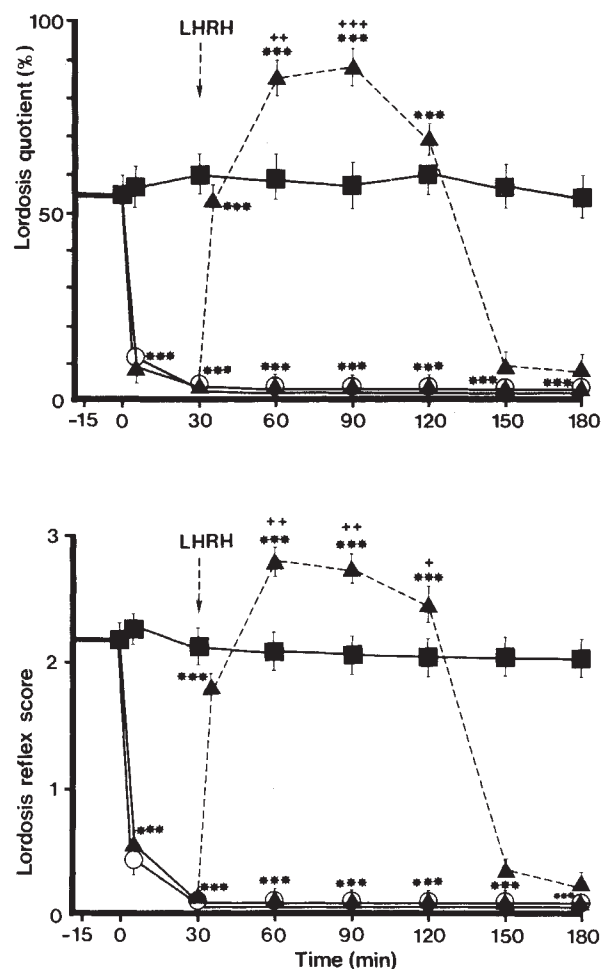


Fig. 3 The effect of saline ($\blacksquare$), CRF ($\blacktriangle$), anti-ACTH antiserum+CRF ($\circ$) and anti-ACTH antiserum+CRF+LHRH ($\blacktriangle$) infusions into the mesencephalic central grey on lordosis behaviour in oestrogen-primed female rats. Each time point denotes the mean $\pm$ s.e.m. of 10 rats. Asterisks *** ($P < 0.001$, Student's *t*-test) denote statistically significant differences between groups (CRF versus saline; anti-ACTH antiserum+CRF versus saline; LHRH versus anti-ACTH antiserum+CRF). Crosses + ($P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$) indicate significant differences between LHRH versus saline.

(D.J.S.S., in preparation). Thus, it seems likely that in the present study, the antiserum not only neutralized the β -endorphin released in response to CRF but also overcame the intrinsic β -endorphin inhibition of LHRH release from LHRH terminals in the MCG; the enhanced LHRH secretion was able to potentiate lordosis above control values. This participation of LHRH in the CRF regulation of lordosis is supported by the finding that infusion of LHRH (100 ng; 0.08 nmol) into the MCG 30 min after CRF (when lordosis was abolished) promptly overcame the CRF inhibition of lordosis. This facilitation occurred within 5 min and lasted for 90 min even in the presence of CRF (Fig. 3). CRF could act directly on LHRH terminals in the MCG to inhibit LHRH release; however, these results suggest that CRF may inhibit lordosis via the enhanced β -endorphin inhibition of LHRH release and that LHRH overcame the blockade of lordosis induced by β -endorphin released in response to CRF. Although such a mechanism seems possible, a direct action of CRF on LHRH neurones or LHRH-sensitive neurones cannot be disregarded. Microiontophoretic studies should confirm such possible neurotropic effects of CRF.

To demonstrate a physiological role for CRF in the regulation of female sexual behaviour, its effects were assessed in the oestrogen-progesterone-primed ovariectomized female rat. CRF (0.3 nmol) was infused into the ARC-VMH of animals

($n = 11$) injected 44 h previously with 5 μg oestradiol benzoate. After the infusion of CRF, they were immediately injected subcutaneously with 500 μg progesterone and tested for sexual receptivity 4 h later. Compared with the saline control (lordosis quotient (LQ) 89.3 ± 2.9 (mean $\pm$ s.e.)) the infusion of CRF (LQ 14.2 ± 4.9) significantly ($P < 0.001$, Student's t -test) suppressed lordosis behaviour. A similar suppression of lordosis behaviour was seen when CRF was infused into the MCG (saline, LQ 81.5 ± 3.0 ; CRF, LQ 20.0 ± 5.7 ; $P < 0.001$, $n = 10$, Student's t -test).

The results of this study may support the recent evidence that i.c.v. injections of vasopressin can inhibit sexual behaviour in the female rat³². Vasopressin is known to possess intrinsic CRF biological activity and can potentiate CRF activity of hypothalamic extracts and CRF(1–41) *in vitro*^{33,34}. However, our studies not only provide the first demonstration of neurally-placed CRF in the control of a specific behavioural response but also suggest that CRF neurones could communicate to β -endorphin/ACTH-secreting neurones of the CNS for the neurotransmission of signals necessary not only for the regulation of female sexual behaviour but possibly for a variety of other behavioural responses.

It is unclear whether β -endorphin and ACTH could be co-released from the same neurone in response to CRF or whether the CRF-responsive neurone has the ability to dampen the release of one in preference to the other. However, the possibility that CRF can control female sexual behaviour by a direct action or via β -endorphin and ACTH and by the possible interactions with LHRH implicate CRF as a peptide with extreme integrative power to coordinate external and internal signals to achieve the optimal level of lordosis. Moreover, this proposal of a circuit of four peptides in one CNS site interacting to control one specific behavioural response provokes complex physiological and pharmacological questions regarding neuronal regulation of peptide release or synthesis and interactions at the receptor level.

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Evidence for opiate receptors on pituitary cells

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A hypothalamo-neurohypophyseal enkephalinergic pathway has been described¹ and the pars nervosa of the rat pituitary contains enkephalin-like material² which may coexist in vasopressin and oxytocin terminals³. At the level of the pars nervosa itself, stereospecific opiate receptors with properties very similar to those of brain receptors have been described⁴, and opiates have been shown to inhibit the release of both vasopressin and oxytocin^{5–8}. The location of the opiate receptors involved has been presumed to be pre-terminal on the neurosecretory fibres. Using an autoradiographic technique to visualize opiate receptors, however, we now report that destruction of the neurosecretory fibres following pituitary stalk section does not result in a significant change in the neural lobe opiate receptor population. This suggests that the opiate receptors within the neural lobe may be present on pituitary cells rather than on neurosecretory fibres.

Opiate receptors were localized at the light microscopic level using an *in vitro* autoradiographic technique⁹. High densities of opiate receptors were found in the pars nervosa, with lower densities present in the pars intermedia and the pars distalis (Fig. 1, Table 1).

Stalk sections were performed by stereotaxic placement of a 2-mm blade through the pituitary stalk. The success of this procedure was assessed by measuring the water intake of the rats on four successive days between 10 and 13 days post-operatively. We found a good correlation between the vasopressin content of the pars nervosa, as measured by radioimmunoassay¹⁰, and the mean daily water intake ($r = 0.74$, $P < 0.01$, $n = 25$). A water intake of over 200 ml per kg per day was always associated with $>95\%$ decrease in vasopressin concentrations from the mean level of 487 ± 64 mU per gland ($\pm$ s.e.m., $n = 10$). Only rats with a water intake of over 200 ml per kg were assumed to have successful pituitary stalk transections and were used for further opiate receptor studies.

There was no significant change in the ³H-etorphine binding concentrations of the pars intermedia or pars distalis either 2 or 4 weeks after pituitary stalk section. The pars nervosa, however, showed a 68% increase in opiate receptor concentration at 2 weeks and a 70% increase in opiate receptor concentration at 4 weeks after the stalk section. Volumetric analysis of

Table 1 Effect of pituitary stalk section on the distribution of specific ³H-etorphine binding sites in rat pituitary gland

Study no.	Time after stalk section	Pars distalis	Pars nervosa	Pars intermedia
1	Controls	2.7 ± 0.07	10.6 ± 0.29	4.9 ± 0.2
	2 Weeks	3.5 ± 0.31	$17.8 \pm 1.61^*$	5.7 ± 0.45
2	Controls	1.9 ± 0.6	9.62 ± 1.30	2.05 ± 0.5
	4 Weeks	1.6 ± 0.16	$16.4 \pm 0.81^*$	1.66 ± 0.8

Nonspecific binding levels were defined using 1 μM levorphanol and represented: study 1, 4.1 ± 0.2 grains per $400 \mu\text{m}^2$ ($n = 6$); study 2, 5.0 ± 0.46 grains per $400 \mu\text{m}^2$ ($n = 6$). Grains were counted six times in each animal in three different slides, and nonspecific binding was subtracted from each density measurement to show the distribution of specific binding sites. Values are mean $\pm$ s.e.m. ($n = 6$ for all groups.)

* $P < 0.01$.

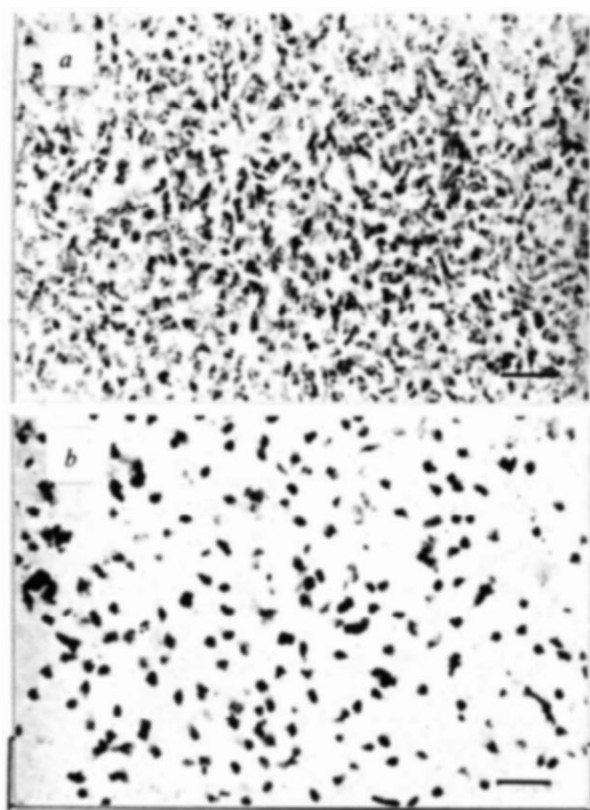


Fig. 1 Dark-field photomicrograph of the distribution of opiate receptors within the pituitary glands of a control animal (a) and 2 weeks after pituitary stalk transection (b). Sections are cut in a coronal plane and show the pars nervosa surrounded by a rim of pars intermedia which is in turn surrounded by pars distalis. Scale bar, 150 μ m. Animals were killed by cervical dislocation. The pituitary glands were rapidly dissected and frozen and 13 μ m thick sections were cut on a cryostat microtome. The sections were thaw-mounted onto cooled subbed slides and incubated in 2 nM 3 H-etorphine ([15,16- 3 H]etorphine; specific activity 41.4 Ci mmol^{-1} , Amersham) at 25 $^{\circ}\text{C}$ for 20 min in 0.17 M Tris-HCl pH 7.4, given two 5-min washes in ice-cold buffer and dried on a stream of cold dry air. Nonspecific binding was assessed in parallel incubations with 1 μ M levorphanol. Emulsion-coated coverslips (Ilford K5) were apposed to the slides and the whole assemblies stored for 6 weeks at 4 $^{\circ}\text{C}$ in the dark. Autoradiographs were subsequently developed with Kodak D19, fixed and the tissue sections processed histologically.

the pituitary gland following stalk section has previously demonstrated a shrinkage of the pars nervosa^{11,12}, and thus it was important to quantitate this change. The neural lobes had diminished in volume by 65% at 2 weeks and 47% at 4 weeks (Table 2). The controls in the 4-week study had greater basal neural lobe volumes and, interestingly, the final neural lobe volumes of both groups were very similar.

Within 10 days of stalk transection, neurosecretory axons have disappeared from the neural lobe, leaving glial cells, collagen fibrils, fibroblasts and capillaries¹³, and examination of the pituitaries used in the present study confirmed the occurrence of considerable gliosis (Fig. 2). However, to be sure that the opioid binding sites did not respond to residual small fragments of neurosecretory axons still left at 2 weeks, the further study was performed at 4 weeks after stalk transection. This provided essentially similar results and even when we account for the very marked shrinkage of the neural lobe, there is only a 41% decrease in the total number of opiate binding sites at 2 weeks and a remarkable 7% at 4 weeks. Thus, despite the loss of neurosecretory axons and immunoreactive vasopressin, there is considerable preservation of opioid binding sites, sug-

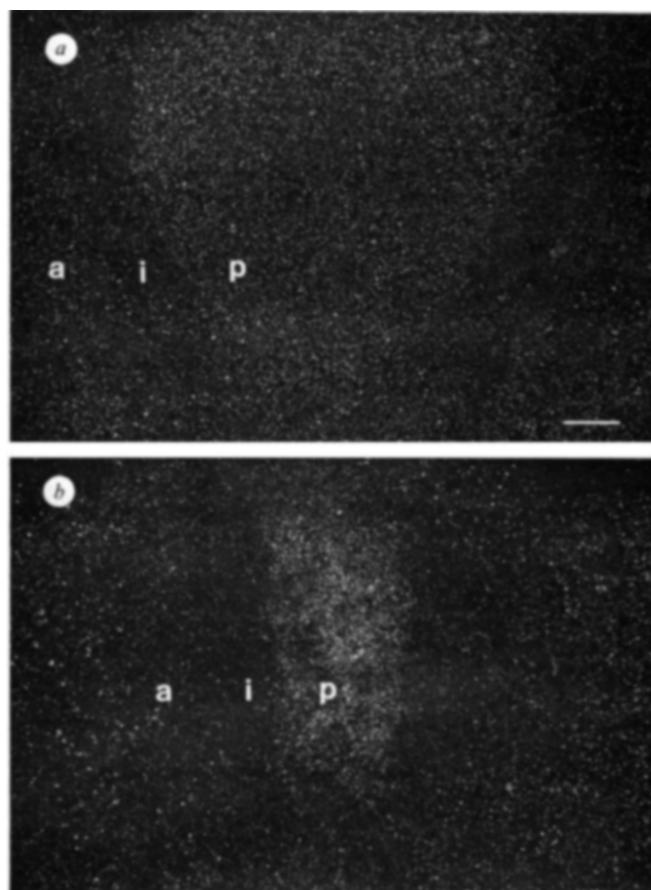


Fig. 2 Nissl stain of pars nervosa from rat 2 weeks after pituitary stalk transection (a) and a control rat (b). Scale bars, 50 μ m.

Table 2 Volumetric measurement of pars nervosa

Study no.	Time after stalk section	Neural lobe volume ($\text{mm}^3 \pm \text{s.e.m.}$)
1	Controls	0.62 ± 0.04 ($n = 4$)
	2 weeks	0.22 ± 0.04 ($n = 5$)
2	Controls	0.43 ± 0.03 ($n = 6$)
	4 weeks	0.23 ± 0.02 ($n = 5$)

Fresh pituitaries were dissected out, frozen onto cryostat chucks and cut at a thickness of 13 μ m. Sections were collected and thaw-mounted onto a subbed slide. Sections were stained with cresyl violet, dehydrated to xylene and mounted in DPX (BDH Chemicals). These were viewed under bright-light illumination and the perimeters of the pars nervosa were drawn using a drawing tube. Areas of lobes were obtained using a digitalizing pad attached to a PDP11 computer.

gesting that opioid receptors are present on cells other than neurosecretory fibres.

It has recently been found¹⁴ that Leu-enkephalin immunoreactivity is present in beaded fibres throughout the neural lobe and that these fibres make synaptoid contacts on the soma and processes of the neurohypophyseal glial cells, the pituicytes. The neurohypophyseal neurosecretory axons often terminate within pituicytes¹⁵ and the number of enclosed axons is highest during periods of low secretory activity. If pituicytes can alter the immediate environment of the neurosecretory terminals, they could be an important link in the control of neurohypophyseal hormone release and might mediate the inhibitory effects of opioid peptides on vasopressin and oxytocin release.

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β -Adrenoceptor activation mediates stress-induced secretion of β -endorphin-related peptides from intermediate but not anterior pituitary

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Recently, we reported that adrenaline can stimulate the secretion of immunoreactive β -endorphin in the rat¹. This response is mediated by β -adrenoceptors and requires circulating adrenaline concentrations which are known to occur during stress^{1–3}. We therefore studied whether catecholamines are implicated in the stress-induced secretion of immunoreactive β -endorphin from the pituitary gland. We report here that in rat the β -adrenoceptor antagonist (–)propranolol reduces or abolishes the rapid increase of immunoreactive β -endorphin levels during some stress stimuli (footshock, passive avoidance, restraint) but not during others (ether, formalin, laparotomy). The propranolol-sensitive response is largely prevented by extirpation of the neurointermediate lobe of the pituitary gland but is unaffected by dexamethasone, which inhibits peptide secretion from the corticotroph cells of the anterior lobe. These results suggest that catecholamines activate the release of immunoreactive β -endorphin from the intermediate lobe but not from the anterior lobe of the pituitary gland during certain stress conditions.

β -Endorphin and related carboxy-terminal fragments of β -lipotropin (β -LPH) are produced in the brain and in the corticotroph cells of the anterior lobe and the melanotroph cells of the intermediate lobe of the pituitary gland^{4–7}. These peptides cross-react in radioimmunoassays for β -endorphin. In the corticotrophs immunoreactive β -endorphin (IBE) primarily represents β -LPH and β -endorphin(1–31), the latter being a potent opioid peptide. However, in the melanotrophs several other peptides are produced in addition to β -endorphin(1–31), which are essentially devoid of opiate activity. These include acetylated β -endorphin(1–31), β -endorphin(1–27), β -endorphin(1–26) and non-acetylated forms of the latter two peptides^{5,7}. The biological activities of these peptides are unknown. However, as the secretion of IBE from the pituitary gland is stimulated during stress^{8–10} it is speculated that β -endorphin and related peptides may have a role in adaptation to life-threatening events.

Female Wistar rats were handled and carefully adapted to the experimental procedures as described in Fig. 1 legend. Rats were decapitated 2 or 4 min after the start of stress exposure and trunk blood was collected in cold tubes containing heparin. Aliquots (1.0–1.5 ml) of plasma were extracted by using Vycor glass powder^{11,12}. The extraction efficiency of synthetic human β -endorphin was $75.0 \pm 1.2\%$ ($n = 12$) and the data have been

corrected accordingly. IBE was quantitated by using a radioimmunoassay in which synthetic human β -endorphin served as a standard^{12,13}. By use of this assay, peptides with chromatographic behaviour similar to that of human β -LPH, β -endorphin(1–31) and β -endorphin(1–27) can be detected in acetic acid extracts of anterior and neurointermediate lobes of rat pituitary glands as well as in plasma extracts of stressed rats. Note that β -LPH represents a small fraction (18%) of IBE in plasma of rats subjected to restraint stress.

Exposure of rats to stress stimuli (see Fig. 1) that are known to activate the pituitary–adrenal system also evoked a quick increase in circulating IBE, with peak concentrations 2–4 min after initiation of stress exposure. These observations support and extend those of others^{8–10,14,15} and strongly indicate that secretion of IBE is an integral part of the acute endocrine response to stress. The increase in plasma IBE to some stress stimuli, such as footshock, passive avoidance or restraint, was diminished or abolished after administration of the β -adrenoceptor blocking agent, (–)propranolol (Fig. 1). This effect is probably due to interaction between propranolol and β -adrenoceptors, as the (+)stereoisomer, which has a much lower affinity for β -adrenoceptors¹⁶, was ineffective in preventing the IBE response to the stress stimuli. These results provide the first clear evidence for a physiological role of β -adrenoceptors in stress-induced IBE secretion.

In contrast to the stimuli that lead to a propranolol-sensitive IBE response, other stressors such as exposure to ether vapour or injection of formalin and laparotomy under Nembutal anaesthesia, induced an increase in circulating IBE that was not affected by (–)propranolol, even at higher doses (up to 10 mg per kg). Thus, we can discriminate between two categories of stress stimuli: those that lead to a propranolol-sensitive IBE response, and those that lead to a propranolol-insensitive IBE response. Although the amplitude of the IBE responses varies greatly from stimulus to stimulus, the differences between propranolol-sensitive and propranolol-insensitive responses appear not to be associated with the amplitude of the IBE response (Fig. 1).

Recently, we have demonstrated that propranolol prevents the increase in plasma IBE concentrations in response to intravenous (i.v.) administration of adrenaline. As most of the circulating IBE after administration of adrenaline originates from the intermediate lobe cells of the pituitary^{12,17}, which are known to possess β -adrenoceptors^{18,19}, we hypothesize that propranolol may prevent the effect of adrenaline by competing for these β -adrenoceptor sites.

Therefore, it was interesting to consider whether the differences in sensitivity to propranolol of stress-induced IBE secretion were related to the source of IBE. Plasma IBE as measured after restraint or formalin stress is accounted for by β -LPH- and β -endorphin-like peptides, in agreement with the findings of others¹⁴. In addition to β -LPH, which is produced predominantly by the corticotrophs, peptides related to β -endorphin are secreted from the corticotroph cells of the anterior lobe and from the melanotroph cells of the intermediate lobe^{9,10}. Thus, both the anterior and intermediate lobe may contribute to the IBE response to stress. In further experiments we studied the origin of IBE secreted in response to restraint and formalin stress by preventing peptide secretion from either the corticotrophs or the melanotrophs. At the dose (250 μ g per kg subcutaneous, s.c.) and time interval (16 h) used in the present experiments dexamethasone does not affect the intermediate lobe activity, as indicated by normal immunoreactive α -melanophore stimulating hormone (α -MSH) responses to various stressors including restraint and formalin stress²⁰. At this dose and time interval, dexamethasone completely prevents the response of the corticotroph cells as indicated by a complete blockade of the immunoreactive ACTH (ACTH₄) response to restraint and formalin stress²⁰. The amplitude of the suppressive effect of dexamethasone on the IBE response is dependent on the nature of the stress stimulus: the increase in plasma IBE in response to formalin (propranolol-insensitive) was reduced by ~75%, while the increase in plasma IBE in response to restraint

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was hardly affected (Table 1). As a complementary model, rats were used that had been subjected to extirpation of the neurointermediate lobe 4 weeks before stress exposure. Such neurointermediate-lobectomized rats showed a nearly normal IBE response to formalin, but the IBE response to restraint stress was reduced by ~70% (Table 1). Thus, these results indicate that during stress leading to a propranolol-insensitive response, IBE is secreted from the corticotroph cells predominantly. Con-

versely, stressors producing a propranolol-sensitive response induce secretion of IBE from the melanotroph cells predominantly. Our results are supported by the observations of Przewlocki *et al.*²¹, who reported a reduced IBE response to footshock in rats 3 weeks after neurointermediate-lobectomy.

The types of stressors that lead to a propranolol-sensitive IBE response are usually considered to induce a high degree of emotionality, and are known to induce a rapid secretion of

Fig. 1 Effect of (+) and (-)propranolol on plasma IBE concentrations in unstressed rats (open blocks) and rats exposed to footshock (a), passive avoidance (b), restraint stress (c), ether (d), formalin (e) and laparotomy (f). Data represent mean $\pm$ s.e.m. ($n=7-8$). * $P<0.01$, ** $P<0.001$ by analysis of variance for stressed (-)propranolol-treated rats versus stressed (+)propranolol-treated rats.

Methods: Female Wistar rats (140–160 g) were housed two per cage in an air-conditioned room at 21–22 °C for at least 5 days before the experiment. Food and water were freely available. The room was entered twice daily by the experimenter only for adaptation procedures for 3–4 days before the actual experiment. On the day of the experiment, saline, (+) or (-)propranolol (2.5 mg per kg) was given intraperitoneally (i.p.) and 40 min later the rats were transferred to an adjacent room and immediately exposed to a stress stimulus. All experiments were done between 9.00 a.m. and 1.00 p.m. **a, Footshock.** Adaptation: rats were injected i.p. with 1 ml 0.9% NaCl, transferred in their home cage to an adjacent room, then each rat was placed in one of the compartments of a shock-box (a metal box with stainless steel grid floor, containing two compartments of 25 $\times$ 25 $\times$ 35 cm each). Experiment: rats were placed in the shock-box, then exposed to an unescapable footshock (50 Hz, 0.5 mA for 2 s). After 2 min the rats were removed from the shock-box, replaced in their home cage and transferred to another room for decapitation. Control rats were treated identically, but no electric shock was applied. **b, Passive avoidance.** Adaptation: rats were injected i.p. with 1 ml 0.9% NaCl and transferred in their home cage to an adjacent room. The rats were then placed in pairs on the entrance grid of a passive avoidance box. When both rats had entered the dark compartment, the door was closed. They were allowed to stay in the dark compartment for 3 min and were then placed back in their home cage. Entrance latencies were <10 s. On the fourth day, one group of rats was treated as above, while another was exposed to an electric footshock (50 Hz, 0.5 mA for 2 s) after entering the dark compartment. Experiment: 1 day after the shock, rats were placed again on the entrance grid and 2 min later, irrespective of whether or not the animals had entered the dark compartment, they were replaced in their home cage and transferred to an adjacent room for decapitation. **c, Restraint.** Adaptation: rats were injected i.p. with 1 ml of 0.9% NaCl. Experiment: on the day of the experiment rats were transferred in their home cages to an adjacent room and placed in front of a restraint box (inner size 14.5 $\times$ 6.4 $\times$ 5.4 cm). After the rats had entered spontaneously, the boxes were closed for 2 min. The rats were then removed from the boxes, replaced in their home cage and transferred to another room for decapitation. Rats that were decapitated within 10 s after transfer from an adjacent room served as a control. **d, Ether.** Adaptation: rats were injected with 1 ml 0.9% NaCl. Experiment: rats were transferred in their home cage to an adjacent room and were immediately placed in a container (25 $\times$ 35 $\times$ 12 cm) and exposed to ether vapour for 2 min. The rats were then placed back in their home cage for 2 min and decapitated. Rats that were decapitated within 10 s after transfer from adjacent room served as controls. **e, Formalin.** Adaptation: rats were injected i.p. with 1 ml of 0.9% NaCl. Experiment: rats were anaesthetized with sodium pentobarbital (40 mg per kg i.p.) 25 min after administration of saline, (+) or (-)propranolol. Fifteen min later, 1 ml of 0.4% formalin in 0.9% NaCl was injected i.p. and rats were decapitated 4 min later. **f, Laparotomy.** Adaptation: rats were injected i.p. with 1 ml 0.9% NaCl. Experiment: rats were anaesthetized with sodium pentobarbital (40 mg per kg i.p.) 25 min after administration of saline, (+) or (-)propranolol. After 15 min the abdominal wall was cut and the intestines were agitated. The decapitation was performed 4 min after the laparotomy. Control rats were treated identically, but no surgery was performed.

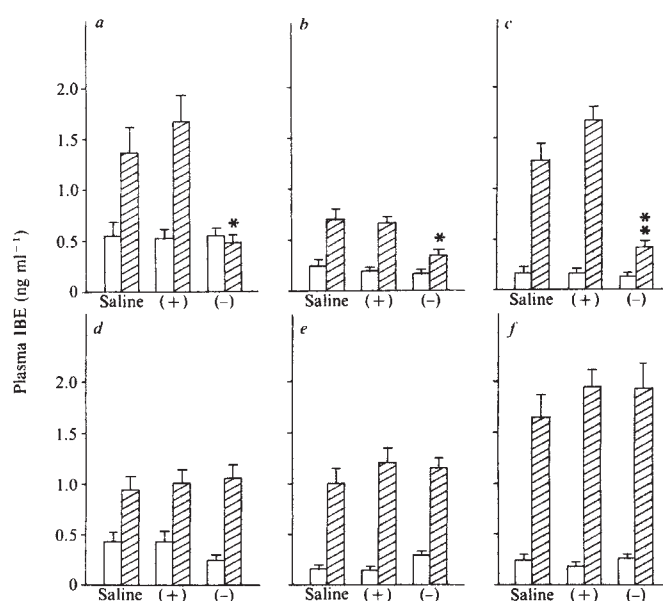


Table 1 Effect of formalin or restraint stress on plasma levels of IBE in intact, dexamethasone-treated and neurointermediate-lobectomized rats

Plasma β -endorphin immunoreactivity (ng ml ⁻¹)						
	Basal	Formalin	Δ	Basal	Restraint	Δ
Saline	0.14 $\pm$ 0.02	1.62 $\pm$ 0.10	1.48	0.22 $\pm$ 0.02	1.54 $\pm$ 0.21	1.32
Dex	0.17 $\pm$ 0.01	0.52 $\pm$ 0.05	0.35	0.10 $\pm$ 0.07	1.22 $\pm$ 0.18	1.12
Sham	0.26 $\pm$ 0.02	1.43 $\pm$ 0.10	1.17	0.22 $\pm$ 0.04	0.94 $\pm$ 0.12	0.72
NIL	0.09 $\pm$ 0.02	0.98 $\pm$ 0.12	0.89	0.11 $\pm$ 0.02	0.32 $\pm$ 0.08	0.21

Saline (0.9% NaCl in water) or dexamethasone phosphate-containing saline (Dex; 250 μ g per kg) was given s.c. 16 h before stress exposure. Neurointermediate lobeectomy (NIL) was performed via the parapharyngeal approach under Hypnorm anaesthesia. After midsagittal cleavage of the anterior lobe, the neurointermediate lobe was removed by suction. Sham-operated rats were treated identically but the neurointermediate lobe was not removed. Two weeks after surgery the rats were exposed to ether vapour for 1 min and 20 min later a blood sample (1.0 ml) was obtained from a lateral tail vein. Plasma was used for the determination of corticosterone by fluorimetric assay²² and α -MSH by radioimmunoassay²³. Only those NIL animals were used that showed undetectable levels of circulating α -MSH and a normal corticosterone response to ether stress (30–40 μ g per 100 ml). Four weeks after surgery the rats were subjected to formalin (propranolol-insensitive) stress or restraint (propranolol-sensitive) stress as described in Fig. 1 legend. Data represent mean $\pm$ s.e.m. ($n=6-8$). Note that dexamethasone did not affect basal IBE levels, whereas neurointermediate lobeectomy caused a consistent reduction.

* $P<0.001$; † $P<0.05$; ‡ $P<0.01$; NS, not significant ($P<0.05$) by analysis of variance.

adrenaline². The circulating concentrations of adrenaline that are found during such stress conditions are alone sufficient to induce IBE secretion¹. Therefore, it is tempting to suggest that the sympatho-adrenomedullary discharge of adrenaline may, via interaction with β -adrenoceptors in the intermediate lobe, stimulate the secretion of IBE in response to certain types of stress.

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Functional identity between murine γ interferon and macrophage activating factor

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We have previously suggested that two lymphokine activities—macrophage activating factor (MAF) and γ interferon (IFN- γ)—are mediated by the same molecule^{1,2}. Striking similarities were noted in their cellular biosynthesis, rate of inactivation with acid treatment and heating, and elution profiles on Sepharyl S-200. In addition, highly specific polyclonal antibodies to murine IFN- γ neutralized MAF and IFN- γ to a similar degree. However, definitive studies require a pure product and we now report that murine IFN- γ that had been cloned and expressed in a simian nonlymphoid cell line shows MAF activity. But it is not yet known whether IFN- γ is responsible for all the MAF activity in media conditioned by T cells as the possibility for MAF heterogeneity remains.

Recent experimental evidence indicates that macrophage activation for potent tumour cytotoxicity requires at least two agents (signals) acting on the macrophage in a particular sequence³. The first signal (MAF released from stimulated T lymphocytes) increases macrophage sensitivity to the effects of a second activating signal. Our earlier observations that numerous type I interferon inducers (bacterial lipopolysaccharide (LPS), heat-killed *Listeria monocytogenes*, muramyl dipeptide, mycoplasma double-stranded RNA, poly(I).poly(C), and pyran copolymer) have second signal activity^{4,5}, led us to

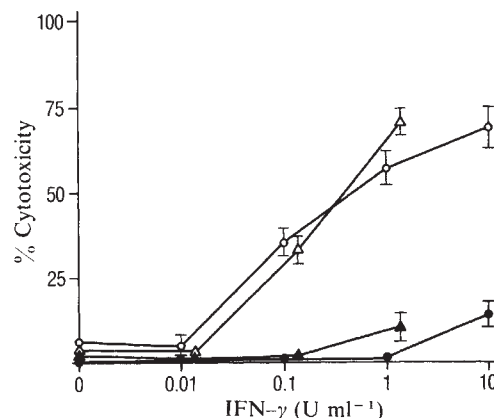


Fig. 1 Synergistic activation of tumour cytotoxicity in CF1 mouse peritoneal macrophages by combinations of LPS and either murine splenic MAF or recombinant murine IFN- γ . The data represent the mean $\pm$ s.e. of three separate experiments. ●, Recombinant murine IFN- γ ; ▲, murine splenic MAF; ○, recombinant murine IFN- γ plus LPS at 0.1 μ g ml⁻¹; △, murine splenic MAF plus LPS at 0.1 μ g ml⁻¹. Both splenic MAF and recombinant IFN- γ were expressed in antiviral units determined by the plaque reduction method using the mouse L cell-vesicular stomatitis virus system. Monolayers of macrophages in 16-mm wells were exposed to these different treatments for 24 h at 37 °C. The macrophages were then overlaid with 4×10^4 P815 mastocytoma cells contained in 1 ml of RPMI-1640 medium supplemented with 20% heat-inactivated (56 °C for 30 min) fetal calf serum and gentamicin at 50 μ g ml⁻¹. All cultures were incubated at 37 °C in an atmosphere of 5% CO₂ in air, and viable tumour cells were counted after 48 h with a haemocytometer. The ratio of macrophages to target cells was approximately 10:1 at the beginning of each experiment. The percentage growth inhibition of P815 cells caused by macrophage-drug interaction was calculated by comparison with P815 cells growth in the presence of normal resting macrophages.

the finding that highly purified preparations of murine α and β interferon themselves act as second signals⁵.

We have previously reported that murine MAF and murine interferon share many physicochemical properties and suggested that these lymphokine activities may be mediated by the same molecule^{1,2}. Some reports on the investigations of IFN- γ and MAF have been confirmatory^{6–8}, but others have been contradictory^{9–16} to our results. With the successful cloning of murine IFN- γ by P. W. Gray (Genentech Inc.), a source of pure IFN- γ was available for unambiguous determination of macrophage activation and antiviral activities. A cDNA sequence coding for murine IFN- γ was expressed in the COS monkey cell line¹⁷. For comparison with the recombinant IFN- γ murine MAF was produced by stimulating BALB/c splenocytes with insoluble (Sephadex-bound) concanavalin A as described previously⁵. The recombinant IFN- γ and splenic MAF were compared for their ability to prime macrophages for cytotoxicity. The results are given in Fig. 1. At roughly equivalent IFN- γ antiviral titres, the recombinant murine IFN- γ produced similar synergy with LPS compared to the activity of murine splenic MAF with LPS. In contrast, conditioned media from the normal COS cell line did not influence macrophage functional activity. Type I interferon preparations have previously been shown to lack MAF priming activity⁵.

The preceding experiments demonstrated the activation of macrophage-mediated cytotoxicity by combinations of LPS and either splenic MAF or recombinant IFN- γ when both agents were incubated simultaneously with peritoneal macrophages. Other studies have shown that macrophages incubated first with suboptimal dilutions of MAF and then exposed to LPS also demonstrated potent tumour cytotoxicity^{3,5}. We wished to examine whether recombinant IFN- γ provides a similar sequence of activation signals to macrophages compared to MAF. As shown in Table 1, IFN- γ behaved identically to splenic MAF. Macrophages had to be either incubated with IFN- γ and LPS simultaneously or treated first with IFN- γ .

Table 1 Sequence of treatment with subthreshold amounts of LPS and MAF or IFN- γ

Sequence and duration of macrophage treatment	Macrophage-mediated cytotoxicity
Untreated peritoneal macrophages	0 $\pm$ 0
MAF* simultaneous with LPS (0.1 μ g ml ⁻¹)	35 $\pm$ 6
MAF followed by LPS (0.1 μ g ml ⁻¹)	
4 h 36 h	48 $\pm$ 6
36 h None	1 $\pm$ 1
LPS (0.1 μ g ml ⁻¹) followed by MAF	
4 h 36 h	1 $\pm$ 1
36 h None	7 $\pm$ 1
IFN- γ (1 U ml ⁻¹) simultaneous with LPS (0.1 μ g ml ⁻¹)	
36 h	59 $\pm$ 4
IFN- γ (1 U ml ⁻¹) followed by LPS (0.1 μ g ml ⁻¹)	
4 h 36 h	55 $\pm$ 3
36 h None	1 $\pm$ 1
LPS (0.1 μ g ml ⁻¹) followed by IFN- γ (1 U ml ⁻¹)	
4 h 36 h	2 $\pm$ 1
36 h None	7 $\pm$ 1

Cytotoxicity was measured by a growth inhibition assay using BALB/c resident peritoneal macrophages and P815 mastocytoma target cells. The data represent the mean $\pm$ s.e. of three separate experiments.

* Spleen MAF was tested at a final IFN- γ antiviral titre of 0.3 U ml⁻¹.

† Period of exposure to indicated agent.

These data suggest that a sequence exists in which IFN- γ or MAF-treated macrophages undergo relatively rapid changes that induce greater responsiveness to LPS as a second signal.

Several T-cell hybridomas^{14,15} and cloned T cells¹³ that produce MAF, but not IFN- γ have been reported. The inability to detect IFN- γ in these preparations may be related to the much greater sensitivity (~50-fold) of the MAF assay than the antiviral assay for IFN- γ . Similarly, IFN- γ induced changes in macrophage Fc receptors or Ia antigens occur with <1 U ml⁻¹ of antiviral activity¹⁸⁻²⁰.

We have previously shown⁵ that IFN- γ differs from type I (α , β) interferon preparations in the ability to prime macrophages for cytotoxic function, but all three interferon types seem to be capable of triggering primed macrophages for cytotoxicity. Similarly, Meltzer²¹ has shown that both priming and trigger signals copurify from lymphokine supernatants. The cellular mechanism of macrophage activation by combinations of IFN- γ and type I interferons is presently under investigation.

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Reciprocal chromosome translocation between c-myc and immunoglobulin $\gamma 2b$ genes

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Specific chromosome translocations have been observed in transformed cell lines of both man and mouse and may be implicated in the origin or maintenance of malignancy¹. In mouse plasmacytomas, translocations have been identified that bring the immunoglobulin α heavy-chain gene (C_{α} , normally located on chromosome 12)² into proximity with c-myc (normally located on chromosome 15)³⁻⁶, c-myc being the mouse cellular homologue of the avian myelocytomatosis virus transforming gene (v-myc). Here we identify a DNA rearrangement in a mouse hybridoma that has brought c-myc close to $C_{\gamma 2b}$ and show that this rearrangement occurred by reciprocal chromosome translocation, as recombinant clones were isolated from the same cell line in which a rearranged variable-region (V_H) gene has been brought close to 5' c-myc sequences. The translocation has resulted in the net loss of 7 base pairs (bp) of chromosome 15 sequence as well as in the presence of an additional base of unknown provenance. This reciprocal translocation was analysed in DNA from a mouse hybridoma cell line but is shown to be characteristic of the X63Ag8 myeloma parent.

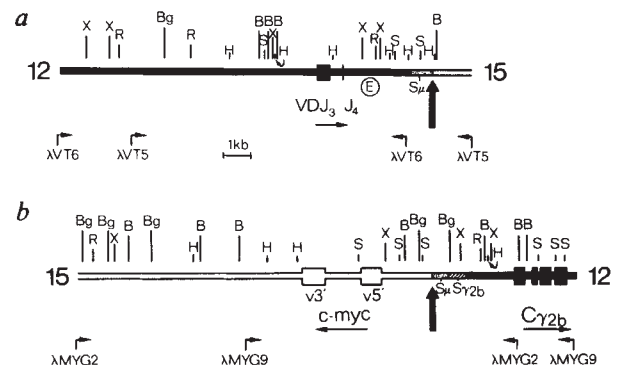


Fig. 1 Maps of the DNA spanning the B1-8 translocated chromosome junctions. *a*, The t(12;15) junction; *b*, the t(15;12) junction. Chromosome 15 sequences are depicted by open lines, chromosome 12 by filled lines, exons by boxes, $S_{\gamma 2b}$ by cross-hatching and S_{μ} by dotting. Vertical arrows mark the chromosome junctions. Only v-myc-homologous exons of c-myc (v_5' and v_3') are indicated, directions of sensible transcription being depicted by horizontal arrows. The designations t(12;15) and t(15;12) may not accord with conventional cytogenetic nomenclature as the orientation of the IgH locus on chromosome 12 is not known with certainty. The location of the immunoglobulin C_H locus transcription enhancer element (E) is taken from ref. 17. Restriction endonuclease cleavage sites are abbreviated: B, *Bam*HI; Bg, *Bgl*II; R, *Eco*RI; H, *Hind*III; S, *Sac*I, X, *Xba*I.

Methods: Partially *Sau*3A-cleaved DNA from the IgM-secreting hybridoma B1-8 μ (refs 20, 21) and from the IgD-secreting line B1-8. $\delta 1$ (which is a descendant of B1-8 μ)²¹ was used to construct genomic libraries using phage λ 1059 (this vector is described in ref. 22). These libraries were screened with either a v-myc probe (the 2.4-kb *Bam*HI fragment of plasmid pMC0)²³ or a 3'- J_H probe (an M13 clone containing an *Xba*I-*Eco*RI fragment located immediately 3' to J_H 4). Positively hybridizing plaques were purified and analysed by restriction mapping. Five distinct v-myc-positive and one J_H -positive phage were obtained from each of the libraries. The restriction maps presented were deduced from phage λ MYG2, λ MYG9, λ VT5 (from the B1-8 μ library) and λ VT6 (from the B1-8. $\delta 1$ library).

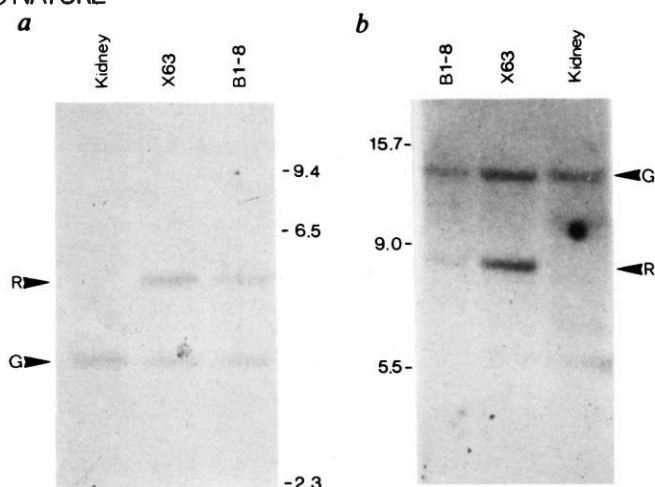


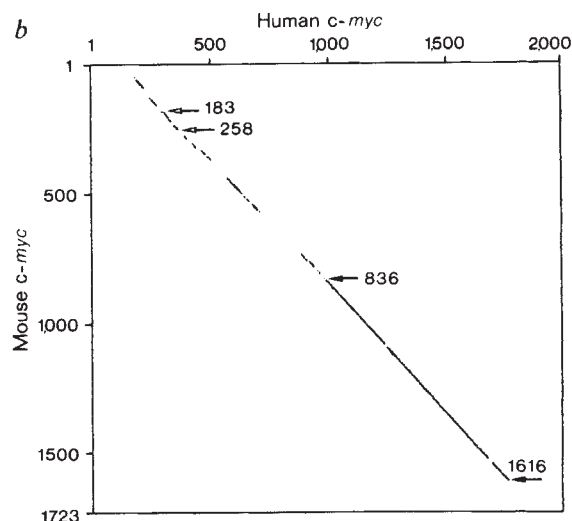
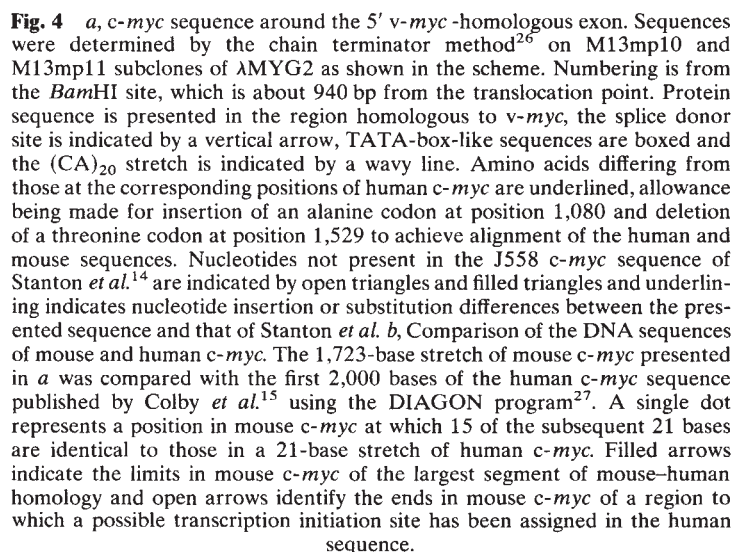
Fig. 3 Southern blot analysis of DNA from B1-8.81, X63Ag8 and BALB/c kidney. *a*, *Hind*III-digested DNA (10 µg) was transferred to nitrocellulose filters and hybridized with the same *v-myc* probe as described in Fig. 1 legend. *b*, *Eco*RI-digested DNA was probed with a nick-translated 2.5-kb λ VT5 restriction fragment extending from the *Bam*HI site in the chromosome 15-derived sequence of λ VT5 to the closest *Bgl*II site in the λ 1059 right arm flanking the mouse DNA insert. Bands due to *c-myc* in the germ line (G) and rearranged (R) configurations are arrowed and the positions of marker fragments are indicated in kb. The sizes of the bands in *a* and *b* due to the rearranged DNA correspond to the restriction maps presented in Fig. 1 for the t(15; 12) and t(12; 15) chromosomes respectively.

During the screening of genomic DNA libraries of the mouse hybridoma cell line B1-8, phage were isolated which contained a rearranged immunoglobulin heavy-chain variable region (V_H) gene. The structures of two clones, λ VT5 and λ VT6, were elucidated by restriction mapping and hybridization with probes specific for the J_H cluster and μ switch region (S_μ) as well as by DNA sequence analysis. The results (Figs 1a, 2a) show that B1-8 contains a V_H segment closely related to that expressed by myeloma MOPC141⁷ rearranged to J_H3 through a putative D segment. This D segment, which bears no obvious homology to the D segments sequenced by Kurosawa and Tonegawa⁸, is 20 bp long and therefore puts the sensible reading frame of the V_H segment out of phase with that of J_H3 . The restriction map of phage λ VT5 indicates that there has been a DNA rearrangement in the area of the switch region and DNA sequence analysis revealed that the rearrangement is due to a translocation involving chromosomes 12 and 15 (t(12;15)), as the sequence flanking S_μ is that found on germ-line chromosome 15 upstream of the *v-myc*-homologous exons of *c-myc* (Fig. 2b).

The structure of the B1-8 t(12; 15) chromosome suggested that it might be the reciprocal of a C_H ; *c-myc* translocation. The B1-8 libraries were therefore screened with a probe specific for *c-myc* and 10 independent phage isolated. The structures of two of these phage, λ MYG2 and λ MYG9, are shown in Fig. 1b. Restriction mapping, hybridization with *c-myc*, C_H and S_{μ} probes as well as DNA sequence analysis demonstrated that λ MYG9 contains a *c-myc* gene adjacent to $C_{\gamma 2b}$ and that the transcriptional orientations of *c-myc* and $C_{\gamma 2b}$ are divergent. The structure of phage λ MYG9 indicates that it carries sequences derived from both chromosome 12 and chromosome 15 and therefore defines a translocated chromosome which we designate t(15; 12) to distinguish it from its reciprocal identified from phage λ VT5 and λ VT6.

These results indicate that a reciprocal (12; 15) chromosome translocation has occurred between the switch region of the immunoglobulin heavy-chain locus on chromosome 12 and sequences upstream of the *v-myc*-homologous region of chromosome 15. The sequences around the chromosome translocation points are presented in Fig. 2b and are compared with

Fig. 2 Sequences around points of DNA rearrangement. *a*, The 3' end of the rearranged V_H gene on λ VT5. The sequence is presented from amino acid residue 78 of the V_H to the 3' end of J_H3 . *b*, The $t(15; 12)$ (i) and $t(12; 15)$ (iii) translocation junctions are compared with the sequence of germ-line chromosome 15 (ii) (this sequence is taken from ref. 14). The dash after the eighth base in (ii) marks a position where Adams *et al.*⁴ but not Stanton *et al.*¹⁴ find a G. Chromosome 12-derived sequences of the $t(15; 12)$ and $t(12; 15)$ chromosomes are indicated by continuous underlining, the region of germ-line chromosome 15 deleted by the translocation is boxed and the adenine of unknown provenance at the $t(15; 12)$ junction is in a dashed box. Postions at which the sequence presented here differs from the sequences determined for the J558 translocated chromosome 15 by Adams *et al.*⁴ and Stanton *et al.*¹⁴ are indicated by a dash over or under the relevant base, respectively. Comparison of the translocated chromosome junctions with germ-line chromosome 12 is not made as the highly repetitious nature of S_μ precludes confident assignment of the points of rearrangement with respect to it. *c*, The S_μ - $S_{\gamma 2b}$ junction in λ MYG9 is compared with the sequences of germ-line S_μ (taken from ref. 24) and germ-line $S_{\gamma 2b}$ (taken from ref. 25). The sequences in germ-line S_μ and $S_{\gamma 2b}$ which are present on λ MYG9 are underlined and the presumed site of recombination boxed. *d*, Deletion within $S_{\gamma 2b}$ on λ MYG9. The sequence of part of the switch region in λ MYG9 is compared with germ-line $S_{\gamma 2b}$. A 46-bp sequence which is repeated in germ-line $S_{\gamma 2b}$ but present only once on λ MYG9 is boxed. The sequence of the λ MYG9 *S*-region presented in *c* is directly adjacent to that presented in *d* and the λ MYG9 $S_{\gamma 2b}$ 46-bp-unit is therefore 30 bases from the S_μ - $S_{\gamma 2b}$ junction.



The B1-8 t(15;12) translocation differs from previously observed *c-myc*; C_H translocations first because $C_{\gamma 2b}$ rather than C_α is adjacent to the rearranged *c-myc* gene and second because S_α rather than S_β flanks the chromosome junction.

However, S_{μ} sequences flank both the rearranged V_H gene on the t(12; 15) chromosome and *c-myc* sequences on the t(15; 12) chromosome; this precludes determination as to whether C_{γ} class switching occurred before or after the translocation. The junction between S_{μ} and $S_{\gamma 2b}$ on λ MYG9 is direct (Fig. 2c) and does not indicate involvement of the switch region of any other γ subclass in the switch to $C_{\gamma 2b}$. A similar structure has been found in the switch region of the expressed $\gamma 2b$ gene of the MPC11 myeloma¹⁰. We also found that λ MYG9 contains a 491-bp deletion of $S_{\gamma 2b}$ sequence (Fig. 2d) which could have occurred by homologous recombination between the 46-bp repeats present in germ-line $S_{\gamma 2b}$. The deletion is unlikely to be a cloning artefact as restriction mapping reveals that it is present in all the independent phages whose inserts span this region.

The phage clones containing the translocated chromosome junctions were obtained from libraries of the hybridoma B1-8 (see Fig. 1 legend). This hybridoma was produced by fusion between a spleen cell from a C57BL/6 mouse and X63Ag8, an 8-azaguanine-resistant variant of the P3 cell line established from the MOPC21 myeloma. MOPC21 is known from cytogenetic evidence to contain a (12;15) translocation¹¹ and, moreover, *c-myc* rearrangements have been detected in several MOPC21-derived hybridomas⁴. The X63Ag8 origin of the translocated chromosomes identified in B1-8 was confirmed by Southern blot analysis with DNA from B1-8, X63Ag8 and kidney (Fig. 3). Bands characteristic of both translocated chromosomes are seen in B1-8 and X63Ag8, but not in kidney (germ-line configuration) DNA. The B1-8 hybridoma has lost expression of the $\gamma 1$ gene of the X63Ag8 parent and possesses only two J_H -hybridizing restriction fragments¹² which can be assigned to the t(12;15) chromosome and to the C57BL/6 chromosome containing the expressed B1-8 heavy-chain gene. Thus, like many somatic cell hybrids, B1-8 has probably undergone chromosome loss after the original fusion event and it is therefore notable that it has retained both the t(12;15) and t(15;12) chromosomes. However, in another X63Ag8-derived hybridoma which has been selected for loss of expression of the X63 $\gamma 1$ gene, Sablitzky *et al.*¹³ have observed loss of both X63Ag8-derived J_H -hybridizing restriction fragments and this may reflect loss of the t(12;15) chromosome.

An exon has been identified in unrearranged *c-myc* that lies upstream of the two *v-myc*-homologous exons ($v5'$ and $v3'$)¹⁴. The chromosome 15 breakpoint of the B1-8 translocation lies within this upstream exon and transcription of the *v-myc*-homologous exons of the t(15;12) chromosome must therefore initiate from a new promoter. In support of this, we have found that X63Ag8 and B1-8 synthesize a 1.9-kilobase (kb) *c-myc* transcript rather than the 2.5-kb transcript characteristic of cells containing only an unrearranged *c-myc* gene (data not shown). As a basis for studying the *c-myc* transcripts from the t(15;12) chromosome, we have determined the DNA sequence in the region of the $v5'$ exon of phage λ MYG2 (Fig. 4a). The region homologous to *v-myc* extends from the ATG codon at position 849 to the splice donor site at position 1,605. Comparison of the mouse *c-myc* sequence presented in Fig. 4a with the human sequence¹⁵ reveals high homology within the $v5'$ exon but this homology falls off sharply 3' to the position 1,605 splice site (Fig. 4b). Interestingly, the part of the $v5'$ exon in which the mouse and human sequences differ most (nucleotides 1,080–1,109) is within a region of *c-myc* which has no homologue in *v-myc*. Upstream of the position 849 ATG codon there are interrupted stretches of homology between the mouse and human sequences. This homology does not include the (CA)₂₀ stretch found in mouse *c-myc* and analysis of the human sequence does not reveal the presence of any other sequence likely to yield a Z-DNA structure.

There are several differences between the sequence we have determined from λ MYG2 and the J558 *c-myc* sequence recently published by Stanton *et al.*¹⁴ (Fig. 4a). Of particular significance are the 3 bases present in our sequence of the $v5'$ exon which are absent from the Stanton *et al.* sequence, as this difference results in a frameshift involving 68 codons. The sequence we present here yields a predicted amino acid sequence which fits better with those deduced from the published *v-myc* and human *c-myc* DNA sequences.

Colby *et al.*¹⁵ have drawn attention to sequences homologous to elements of eukaryotic transcription initiation sites located upstream of the *v-myc*-homologous region of human *c-myc*. At the equivalent position in mouse *c-myc* (around position 210), two 'TATA-box'-like sequences are found but—unlike human *c-myc*—there is nothing in the region closely resembling sequences for RNA cap site and CAATCT upstream element. More recently, Stanton *et al.*¹⁴ have presented data suggesting that *c-myc* transcription from the translocated chromosome 15 in plasmacytoma J558 initiates at three sites in the region of the (CA)₂₀ sequence. Nuclease S₁ mapping with RNA from X63Ag8

gives results consistent with transcription initiating or splicing in at the beginning of the (CA)₂₀ stretch (data not shown).

It has been suggested¹⁶ that the effect of chromosome translocation on *c-myc* transcription might be related to bringing *c-myc* close to a transcription enhancer element located in the region of the immunoglobulin genes. However, although such a transcription enhancer element has indeed been identified in the mouse C_H locus^{17–19}, its position within the large V_H – C_H intron (see Fig. 1) means that, following translocation, it will reside on the t(12;15) chromosome of X63Ag8 and not the t(15;12) chromosome; this enhancer cannot therefore be implicated in the altered transcription of the *v-myc*-homologous exons of *c-myc*.

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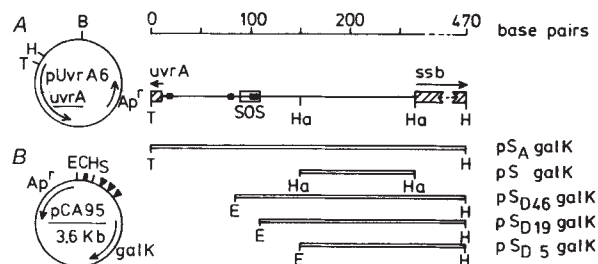
A common regulatory region shared by divergently transcribed genes of the *Escherichia coli* SOS system

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The *Escherichia coli* single-stranded DNA binding protein (SSB) is implicated in DNA replication, recombination and repair¹. On the chromosome, the *ssb* gene is located adjacent to the excision repair gene *uvrA*, but the two genes are transcribed in opposite directions². *uvrA* has been shown to be part of the *E. coli* SOS system by introducing Mud(Ap, *lac*) insertions distal to the regulatory region of the gene in the chromosome³. Recent investigations suggest that SSB is also involved in the SOS response^{1,4,5}. However, because the SSB protein is essential to the cell, the inducibility of the *ssb* gene cannot be investigated by the insertion method. Therefore, we used plasmids harbouring the regulatory region of *ssb* fused to the *galK* structural gene, while leaving an intact *ssb* gene in the chromosome. We show here that expression of the *ssb* gene is dependent on two promoters of which one is damage inducible. Evidence is presented that the divergently transcribed *ssb* and *uvrA* genes are controlled by a common LexA binding site.

Fig. 1 A, Schematic representation of the DNA region between the *uvrA* and *ssb* genes. The ribosome binding site, the Pribnow box and the '-35 region' of the *uvrA* gene have been described elsewhere^{6,7} and are indicated by black boxes. The start of translation of the *ssb* gene has been indicated as described by Sancar *et al.*² B, Vector pCA95 is a derivative of plasmid pKO-1 (ref. 6) and constructed by replacing the small *HindIII*-*EcoRI* fragment of pKO-1 by the small *EcoRI*-*HindIII* fragment of pBR322¹⁴. The fragments that are subsequently introduced in pKO-1 or pCA95 are shown. The construction of the fusion plasmids consisted of the following steps: (1) Ligation of the *HindIII*-*TaqI* fragment of pUvrA6⁶ to the large *Clal*-*HindIII* fragment of plasmid pCA95, giving pS_AgalK. (2) Linearization of plasmid pS_AgalK by *EcoRI*, *Bal31* treatment (see ref. 6), addition of *EcoRI* linkers (5'GGAATTCC) and subsequent ligation of the series of small *EcoRI*-*HindIII* fragments (varying in length over 300–400 bp) to the isolated large *EcoRI*-*HindIII* fragment of pCA95. Procedures followed to purify DNA fragments have been described previously⁶. The exact deletion points of the pS_DgalK plasmids obtained have been determined by DNA sequencing⁶, and are indicated in Fig. 3. (3) Plasmid pSgalK consists of a *HaeIII*-*HaeIII* fragment of pUvrA6 ligated into the *SmaI* site of vector pKO-1. The orientation of the inserted fragment has been determined by DNA sequencing (results not shown). B, *Bam*HI; C, *Clal*; H, *HindIII*; Ha, *HaeIII*; E, *EcoRI*; S, *SmaI*; T, *TaqI*.



The *uvrA* recombinant plasmid pUvrA6 (Fig. 1)⁶ contains the intercistronic region of the *uvrA* and *ssb* genes. A *TaqI*-*HindIII* fragment was inserted in front of the *galK* gene of vector pCA95, resulting in fusion of the regulatory region of *ssb* to *galK* (pS_AgalK, Fig. 1). Measurement of galactokinase levels in strains carrying this fusion plasmid showed increased expression of *galK* on UV or mitomycin C treatment (Fig. 2, Table 1). The *recA*⁺*lexA*⁺ dependency of this phenomenon was demonstrated (1) by the absence of inducibility in a *RecA* background, and (2) by a high, constitutive level in a *Spr* strain

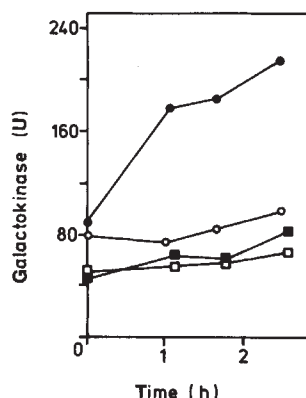


Fig. 2 Kinetics of induction of galactokinase in strain AB1157(*rec*⁺*lex*⁺) harbouring pS_AgalK without UV (○), with UV (●), or pSgalK without UV (□), with UV (■). Early-log phase cultures growing in supplemented M9 media were irradiated with UV (40 J m⁻²). Measurement of galactokinase activity was performed as described previously⁶. The experiment has been repeated 10 times; in each experiment an induction of two- to threefold was observed.

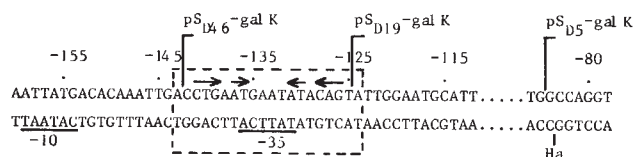


Fig. 3 The DNA sequence of the common regulatory region shared by the *ssb* and *uvrA* genes. The -35 and Pribnow sequences of the *uvrA* gene have been indicated. The coordinates indicated refer to the numbering used by Sancar *et al.*². Deletion points of the pSgalK fusions are shown. In the pS_{D46}galK fusion, the -35 region¹⁵ at position 141–146 (see text) is partially restored by the *EcoRI* linker. The LexA recognition site has been boxed and arrows indicate its symmetrical organization. Ha, *HaeIII*.

and, at 42 °C, in a *Tsl* strain (Table 1). When a smaller fragment (*HaeIII*-*HaeIII*) of pUvrA6 was introduced in front of the *galK* gene (pSgalK, Fig. 1), a substantial level of expression was still measured, but induction by UV or mitomycin C was no longer observed (Fig. 2, Table 1). Apparently, the *HaeIII* fragment contains a non-inducible *ssb* promoter, possibly the one tentatively described by Sancar *et al.*². Moreover, our results indicate that sequences involved in SOS induction of the *ssb* gene are located upstream of the non-inducible promoter (to the left of the *HaeIII* fragment in Fig. 1). The only LexA recognition site detectable in this region is the one involved in repression of the *uvrA* gene^{6,7}. LexA binding to this region may repress the transcription of both the *uvrA* and the *ssb* genes. To test this hypothesis we constructed two deletion plasmids of pSgalK, that differ only in the presence of the LexA binding site (Fig. 1). The galactokinase activities produced by strains harbouring these plasmids are given in Table 1. The inducibility of galactokinase production in bacteria containing pS_{D46}galK and its absence in the case of pS_{D19}galK, clearly demonstrate the

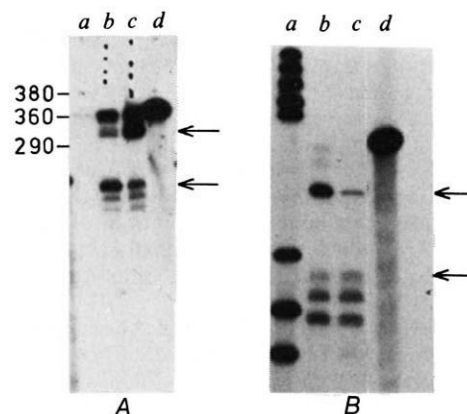


Fig. 4 S₁ mapping¹⁶ of two ³²P end-labelled DNA fragments of different length protected by *in vivo* synthesized transcripts. The *EcoRI*-*HindIII* fragments (Fig. 1) of pS_{D19}galK (A) or pS_{D46}galK (B) were labelled at both ends and hybridized at 46 °C to RNA extracted from unirradiated and UV irradiated (45 J m⁻²) strains AB1157, carrying pS_AgalK. Methods were as described earlier⁶. A, 20% PAA gel; lane a, DNA + S₁; lane b, DNA + RNA - UV; lane c, DNA + RNA + UV; lane d, DNA. The length of labelled DNA fragments run on the same gel, are indicated. B, 8% PAA gel; lane a, labelled *HaeIII* fragments of plasmid pBR322¹⁴ with a length, respectively, of 587, 540, 504, 457, 434, 267, 234 and 213 bp; lane b, DNA + RNA + UV; lane c, DNA + RNA - UV; lane d, DNA. The arrows indicate the position of the two transcripts observed. The experiment has also been done with a probe labelled at one end (*HindIII* site) which gave similar results (not shown).

Table 1 Galactokinase levels with and without inducing treatment

Fusion plasmid	Host	Inducing treatment	Units GalK	After 1 h	Units GalK	After 2 h
				Induced Non-induced		Induced Non-induced
pS _{AgalK}	AB1157	—	90	2.0	84	3.0
	AB1157	mitC	182		252	
	AB1157	—	—	1.1	109	1.1
	AB2463	—	—		120	
	AB1157	—	—	2.0	80	2.0
	DM1187	—	—		162	
	DM511	32 °C	—	2.1	100	2.1
pS _{galK}	DM511	42 °C	—		210	
	AB1157	—	70	1.0	78	1.3
pS _{D46galK}	AB1157	mitC	70		102	
	AB1157	—	86	2.5	112	2.4
pS _{D19galK}	AB1157	mitC	216		266	
	AB1157	—	78	1.0	104	1.2
pS _{D5galK}	AB1157	mitC	78		120	
	AB1157	—	80	1.1	80	1.1
	AB1157	mitC	90		84	

Strains AB2463(*recA*), DM1187(*spr*) and DM511(*tsl*) are derivatives of strain AB1157(*rec⁺lex⁺*)⁹. Growth conditions and measurement of galactokinase levels were as described previously⁶. Mitomycin C (mitC) was added to the cultures to a final concentration of 5 µg ml⁻¹. Units galK are normalized for cell density. (The galactokinase levels measured with plasmid pS_{D5galK} are comparable with that promoted by pS_{galK}, indicating that deletion of the *HaeIII*–*HindIII* fragment in the case of pS_{galK} (Fig. 1) does not influence the expression of galK.)

involvement of the SOS box in the induction of the *ssb* gene (Table 1, Fig. 3).

For other damage-inducible genes, the regulation by LexA can be explained by the location of the SOS box in the promoter region of the corresponding gene. Examination of the DNA sequence around the LexA binding site reveals the presence of a putative promoter (‘–35’: position 141–146; ‘–10’: position 117–122; Fig. 3). Evidence for this promoter was obtained by the identification of an inducible transcript starting about 10 base pairs (bp) downstream of the LexA binding site, by S₁ mapping (Fig. 4). In addition to the inducible one, a shorter transcript can be detected (Fig. 4) which is not inducible. We are at present determining the precise location of this promoter. We conclude that the *ssb* gene is transcribed from both a non-inducible and a damage-inducible promoter, the latter being regulated by the same repressor binding site as the *uvrA* promoter.

The presence of a non-inducible promoter for *ssb* transcription can be explained from the central role of the SSB protein in DNA replication⁸. On the other hand, the inducibility of the *ssb* gene might reflect its presumed role in the regulation of the SOS response. It has been proposed that SSB is particularly involved in the availability of signal molecules necessary for activation of RecA protein⁹. Therefore, the amount of SSB protein is probably critical for the cellular response to DNA damage. This is also demonstrated by the finding that presence of the *ssb* gene on a multicopy plasmid sensitizes wild-type cells to UV¹⁰. Furthermore, it has been reported that both in *ssb* mutants and in cells that overproduce the SSB protein, RecA induction is inhibited¹¹. As RecA also binds to single-strand DNA, both proteins might compete for single-strand regions. The increase in SSB protein after SOS induction might counteract the activation of RecA, subsequently leading to the turn-off of the SOS response.

The finding that the LexA binding site located between two genes can be functional in both directions is novel. Recently, Reed *et al.*¹² have described a *TnpR* repressor, regulating two divergently transcribed genes at a single site in between these genes, and have shown that the *TnpR* repressor acts on both *TnpA* and *TnpR* transcription. Also in this case, the promoters of both genes are overlapping.

Our data seem to contradict the recent results of Salles and co-workers¹³ who found no evidence for induction of SSB after treatment of *E. coli* cells with DNA damaging agents. Their method is based on the immunological measurement of the amount of SSB protein present in the cell before and after treatment. The reason for this discrepancy is unknown and requires further study. One possible explanation might be that the amount of SSB protein detectable by the IRMA method is dependent on the number of SSB binding sites, which increases strongly after treatment of the cells with DNA damaging agents.

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Amplified DNA with limited homology to *myc* cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour

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Amplified cellular genes in mammalian cells frequently manifest themselves as double minute chromosomes (DMs) and homogeneously staining regions of chromosomes (HSRs)^{1,2}. With few exceptions both karyotypic abnormalities appear to be confined to tumour cells^{3,4}. All vertebrates possess a set of cellular genes homologous to the transforming genes of RNA tumour viruses, and there is circumstantial evidence that these cellular oncogenes are involved in tumorigenesis⁵. We have recently shown that DMs and HSRs in cells of the mouse adrenocortical tumour Y1⁶ and an HSR in the human colon carcinoma COLO320⁷ contain amplified copies of the cellular oncogenes *c-Ki-ras* and *c-myc*, respectively. Both DMs and HSRs are found with remarkable frequency in cells of human neuroblastomas^{4,8–15}. We show here that a DNA domain detectable by partial homology to the *myc* oncogene is amplified up to 140-fold in cell lines derived from different human neuroblastomas and in a neuroblastoma tumour, but not in other tumour

cells showing cytological evidence for gene amplification. By *in situ* hybridization we found that HSRs are the chromosomal sites of the amplified DNA. The frequency with which this amplification appears in cells from neuroblastomas and its apparent specificity raise the possibility that one or more of the genes contained within the amplified domain contribute to tumorigenesis.

We performed initial studies with the neuroblastoma cell lines NMB^{8,10} and Kelly (F. G., unpublished), each of which contains HSRs, to look for evidence for amplification of known oncogenes. DNA cleaved with restriction endonuclease *Eco*RI was analysed by agarose gel electrophoresis and Southern blotting in conditions of reduced stringency for sequences homologous to 14 retroviral transforming genes (*src*, *yes*, *myb*, *myc*, *erb*, *rel*, *fps*, *mos*, *fos*, *abl*, *Ha-ras*, *Ki-ras*, *fes* and *sis*). DNA from both cell lines yielded a 2.0-kilobase pair (kbp) *Eco*RI fragment that hybridized with the probe for *v-myc* (Fig. 1). An analogous fragment was barely visible in DNA from normal human fibroblasts and cannot be seen in Fig. 1.

The major *c-myc* locus in human DNA lies within a 13.5-kbp *Eco*RI fragment¹⁶. Because human *myc* has diverged appreciably from the analogous avian gene, we could readily detect the 13.5-kbp fragment with *v-myc* probe only in DNA in which the locus is amplified (COLO320 DNA⁷ in Fig. 1). In addition to the 13.5-kbp and the 2.0-kbp fragments, a number of fragments is detectable with *v-myc* probe in all human cell lines. This result has also been reported by others¹⁶. It shows that the human genome contains a series of sequences related to the *myc* oncogene.

The 2.0-kbp *Eco*RI fragment derived from amplified DNA in neuroblastoma cells is not contained within the major locus of human *c-myc*¹⁶. We explored the nature of the *c-myc*-related sequences in the amplified region by using probes for the 5' and 3' domains of *v-myc* (Fig. 1). The 2.0-kbp *Eco*RI fragment reacted primarily with the probe for the 5' domain. The probe for the 3' domain reacted well with the amplified *c-myc* locus in the DNA of COLO320 cells, but very poorly with the 2.0-kbp *Eco*RI fragment and not with any other fragment from neuroblastoma DNA other than the 13.5-kbp fragment. We conclude that the 2.0-kbp *Eco*RI fragment contains a sequence homologous primarily to the 5' domain of the *myc* oncogene.

For more detailed studies we cloned the *myc*-related sequence from neuroblastoma line Kelly into bacteriophage λ gtWES, and subcloned into pBR322 an approximately 1.0-kbp *Eco*RI-*Bam*HI fragment (Nb-1) that contained the *myc*-related sequence. Other investigators have recently cloned from human cells three sequences related to the 5' end of *myc*¹⁶. It is likely that we have isolated an additional sequence, for the published restriction endonuclease maps are different from the one we have obtained.

We have further analysed the homology of Nb-1 to human *c-myc*. The *c-myc* locus used is an apparently normal allele cloned previously from COLO320 cells (M.S., unpublished results); the restriction endonuclease map of the cloned DNA was found to be identical to that published by other investigators¹⁶. Southern blotting analyses showed that the region of homology is confined to a 1.4-kbp *Sac*I fragment of *c-myc* (Fig. 2A) containing the exon that gave rise to the 5' domain of *v-myc*. This result is not unexpected, as we had previously established (Fig. 1) that a probe specific for the 5' domain of *v-myc* gives a strong signal on Southern blots containing genomic DNA of neuroblastomas. Additional analyses revealed that the homologous region is localized within a 0.35-kbp *Xho*I-*Pst*I fragment of Nb-1 and maps to a 0.4-kbp *Pst*I fragment of *c-myc* (Fig. 2A).

We have sequenced the 0.35-kbp *Xho*I-*Pst*I fragment of Nb-1 and compared the nucleotide sequence with that of the 0.4-kbp *Pst*-I fragment of *c-myc*¹⁷. Inspection of the nucleotide sequences reveals that Nb-1 contains two blocks 74 and 59 base pairs long showing 78% homology to corresponding regions of *c-myc*, and separated by a stretch of 116 nucleotides that bears no apparent relationship to *c-myc* (Fig. 2B). The

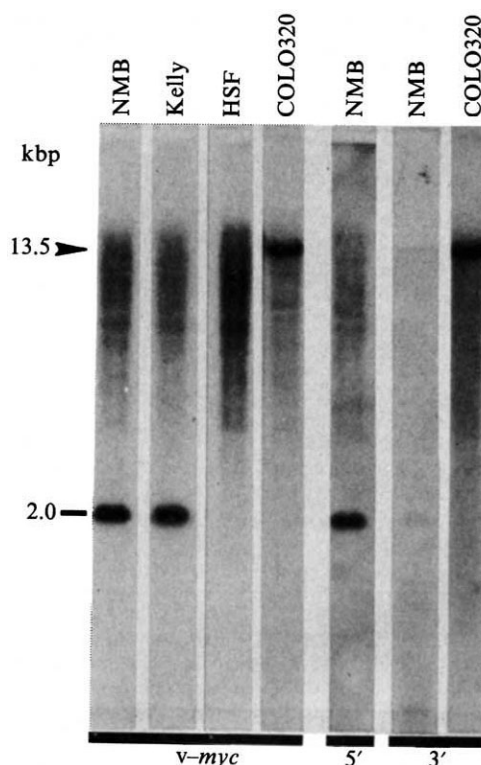


Fig. 1 Identification of amplified DNA related to the *myc* oncogene in human neuroblastoma cells. Bar indicates position of the *myc*-related sequence, arrowhead that of the complete human *c-myc*. NMB^{8,10} and Kelly (F.G., unpublished) are human neuroblastoma cell lines, COLO320 is a line of neuroendocrine cells of a human colon carcinoma²⁵ (for this study the HSR-subline of COLO320 was used in which an apparently normal *c-myc* locus is amplified 16–32-fold⁷). HSF are human skin fibroblasts. Each lane contained 10 μ g DNA. **Methods:** DNA was isolated as previously described⁶, digested with restriction endonuclease *Eco*RI, separated on agarose gels and transferred to nitrocellulose. The filters were hybridized to a radioactive 1.2-kbp *Pst*I fragment of avian myelocytomatosis virus clone MC38 containing *v-myc*²⁶, or to a *Pst*I-*Sal*I or a *Sal*I-*Pst*I fragment containing the 5' and 3' ends of *v-myc*, respectively²⁶. Stringency in the hybridizations was as described earlier⁶ (40% formamide, 0.45 M NaCl, 0.045 M Na-citrate, 41 °C).

Table 1 Amplified DNA detectable by partial homology to the *myc* oncogene in neuroblastoma cell lines and a neuroblastoma tumour

Designation of cells	Tumour	Ref.	HSR on chromosome	DMs	Fold amplification
Kelly	Nb	F.G.*	13p, markers	–	100–120
NGP	Nb	10	4p16, 12q13	–	120–140
NLF	Nb	G.B.*	9q13	–	20–25
CHP-134	Nb	8	6q, 7p	–	20–25
CHP-126	Nb	8, 9	5q33	+	100–120
IMR-32	Nb	13	1p34	–	15–20
NMB	Nb	8–10	13p11	+	100–120
MCN-1	Nb	12	–	+	5–8
AR (tumour)	Nb	This study	?	?	80–100
SK-N-SH	Nb	24	–	–	0
HA-A	Mel	J.T.*	7p	–	0
HA-L	Mel	J.T.*	7p	–	0
Y79	Ret	14	1p34	–	0
COLO320	Carc	25	X†	–	0

Nb, neuroblastoma; Mel, melanoma; Ret, retinoblastoma; Carc, neuroendocrine cells from a carcinoma of the colon. +, – Indicates the presence or absence of HSRs or DMs. The degree of amplification was calculated by blotting different amounts of DNA and by varying the exposure times of the autoradiographs.

* Unpublished results of F.G., G.B. and J.T., respectively.

† C. C. Lin, personal communication.

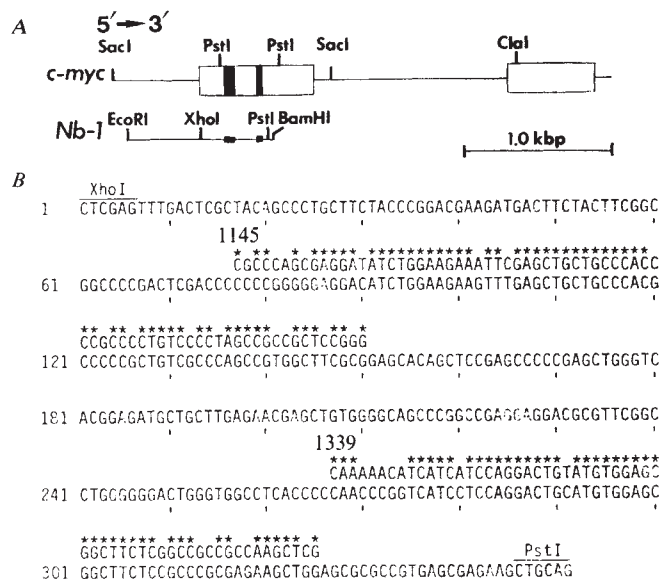


Fig. 2 Analysis of homology between the *myc*-related sequence amplified in neuroblastoma cells and complete human *c-myc*. **A**, Mapping of the *myc*-related sequence to *c-myc*. The map of *c-myc* (upper map) was established by using a recombinant bacteriophage containing the *c-myc* locus isolated from COLO320²⁵ cells (unpublished results of M.S.). Large rectangles symbolize major exons of human *c-myc* according to ref. 17. The *myc*-related sequence was isolated from neuroblastoma line Kelly. Total genomic DNA was partially digested with the restriction endonuclease *Eco*RI and ligated into bacteriophage λ gtWES arms. From $\sim 10^4$ recombinants a recombinant phage was purified that carries the 2.0-kbp *myc*-related sequence. The region related to *myc* was further mapped in bacteriophage and genomic DNA to a 1.0-kbp *Eco*RI–*Bam*HI fragment (Nb-1; lower map), which was subcloned into pBR322 (pNb-1). The region of homology in Nb-1 is confined to a 0.35-kbp *Xho*I–*Pst*I fragment. The precise regions of homology between *c-myc* and Nb-1, determined by nucleotide sequencing, are indicated by solid black rectangles in both diagrams. **B**, Comparison of the nucleotide sequences of the *myc*-related amplified DNA with the homologous region in *c-myc*. Asterisks indicate homology between the *myc*-related sequence amplified in neuroblastoma cells and human *c-myc*; numbers refer to the nucleotide positions in *c-myc* according to ref. 17. Nb-1 was digested with restriction endonucleases *Xho*I and *Pst*I, cloned into bacteriophages M13mp8 and M13mp9, and the nucleotide sequence spanning the *Xho*I–*Pst*I restriction endonuclease sites was determined by the dideoxynucleotide chain termination method²⁷. The nucleotide sequence of the major human *c-myc* locus was taken from ref. 17.

two blocks of homology with *c-myc* represent the only apparent explanation for the hybridization between Nb-1 and the *myc* probe.

A single open reading frame spans the entire sequence illustrated in Fig. 2B. In the regions that are homologous to *c-myc*, the open reading frame encodes amino acid sequences that can be aligned with *c-myc* (data not shown). Moreover, the diverged sequence that separates the two regions of homology is virtually the same length in both Nb-1 and *c-myc*. It seems possible that *c-myc* and the domain represented by Nb-1 diverged from the same distant ancestor. We do not yet know whether the DNA of Nb-1 is derived from a functional gene, nor whether any portion of the DNA amplified in neuroblastoma cells is expressed in either normal or malignant tissues. Studies on the expression of the amplified DNA are now in progress.

We have tested nine neuroblastoma lines for amplification of the region that yields the 2.0-kbp *Eco*RI fragment with the *myc*-related sequence, using Nb-1 as the probe (Fig. 3, Table 1). In addition, we have tested a metastatic neuroblastoma (AR) obtained from a 13-month-old female patient before chemotherapy. All cell lines except one (SK-N-SH) contain DMs and/or HSRs (Table 1); the karyotype of the tumour AR has not been established. Amplification was found in all the

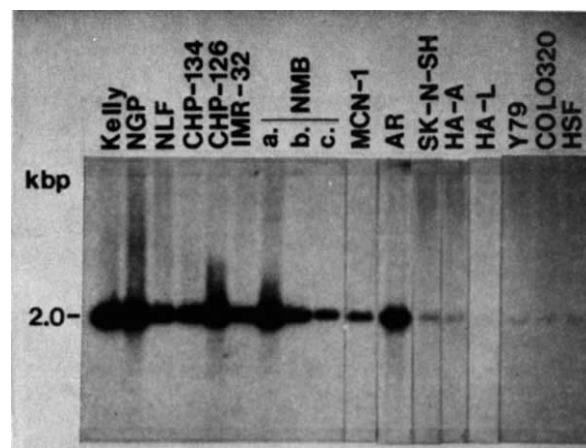


Fig. 3 Survey of amplified DNA in neuroblastoma cells. Neuroblastoma lines tested were Kelly, NGP, NLF, CHP-134, CHP-126, IMR-32, NMB, HCN-1 and SK-N-SH. AR is a neuroblastoma tumour. Y79 is a retinoblastoma cell line, HA-A and HA-L are melanoma cell lines and COLO320 is a neuroendocrine cell line from a colon carcinoma; all of these cell lines carry an HSR. HSF, human skin fibroblasts. Origin and cytological characteristics of the tumour cells are compiled in Table 1. For isolation of DNA from the tumour tissue, a portion of the frozen material was thawed, minced finely on ice with a razor blade and then briefly homogenized in 0.1 M NaCl, 10 mM EDTA, 10 mM Tris-HCl, pH 8.0 using a motor driven glass homogenizer and a Teflon pestle. The subsequent steps were as described for isolation of DNA from cultivated cells⁶. DNA was digested with restriction endonuclease *Eco*RI and analysed by Southern blotting as described for Fig. 1, except that the formamide concentration in the hybridization mixture was 50%. Nb-1 (Fig. 2A) was used as molecular probe. The amount of DNA for each lane was 10 μ g except for lanes b and c, where 1.0 and 0.2 μ g of NMB DNA were applied to achieve dilutions of 10- and 50-fold, respectively.

cell lines showing cytological evidence of gene amplification. The line SK-N-SH, which shows neither DMs nor an HSR, does not contain an amplified *myc*-related sequence. The degree of amplification varied considerably between the different neuroblastoma lines, by 120–140-fold in NGP to 5–8-fold in MCN-1 (Table 1). The DNA from the tumour AR showed approximately 80–100-fold amplification. Several HSR-bearing cell lines established from other types of tumours, such as melanoma, retinoblastoma and colon carcinoma (Fig. 3), showed no amplification of the *myc*-related sequence: the signal obtained in Southern blots was invariably faint and not different from that displayed by DNA from skin fibroblasts. The major *c-myc* locus is not detected by the Nb-1 probe in the conditions of hybridization used, even when amplified as in COLO320 cells. Similar results were obtained when we analysed DNA digested with restriction endonucleases *Hind*III and *Bam*HI (data not shown). Our results show that multiple cell lines derived from neuroblastomas in several laboratories and also tumour tissue from a neuroblastoma share a specific amplified sequence whose abundance is not increased in other tumour cells with karyotypic evidence of gene amplification.

We sought the chromosomal location of amplified DNA in neuroblastoma cells by using hybridization *in situ* with ³H-labelled DNA of Nb-1. Three cell lines have been tested—NGP, NLF and NMB (Table 1)—in all of which autoradiography revealed clustering of grains along HSRs. Figure 4 illustrates representative data for one of the two HSRs (4p) in line NGP. Details of our findings with other cell lines will be reported elsewhere (manuscript in preparation). The number of autoradiographic grains was roughly proportional to the extent of amplification in the cell lines (summarized in Table 1): the strongest signal was obtained with line NGP, weaker signals with lines NMB and NLF. We conclude that the HSRs in neuroblastoma cell lines carry amplified DNA that includes a domain distantly related to *c-myc*.

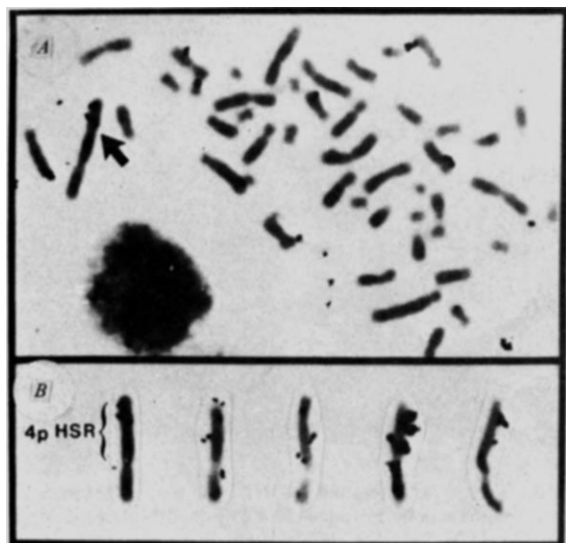


Fig. 4 Chromosomal localization of the *myc*-related sequence amplified in neuroblastoma cell line NGP. **A**, Metaphase spread showing clustering of grains to the HSR on chromosome 4p16 (arrow). **B**, Chromosome 4 from five different metaphase spreads to which labelled Nb-1 was hybridized. ^3H -labelled Nb-1 was used as molecular probe. The *in situ* hybridization, autoradiographic and photographic procedures have been described elsewhere²⁸.

The HSRs in the various neuroblastoma cell lines occupy different chromosomal locations (see Table 1). Because it is likely that the DNA represented by Nb-1 comprises one or very few copies in normal cells, we suggest that amplification of the DNA is followed by translocation to any of numerous chromosomal sites. The issue can be resolved conclusively by mapping the DNA of Nb-1 on the chromosomes of normal cells (work in progress).

Other investigators have recently shown by DNA-mediated gene transfer using NIH/3T3 cells as recipients that the neuroblastoma line SK-N-SH carries a transforming gene¹⁸ related to Ha- and Ki-ras (*N-ras*)¹⁹. It is possible that at least two distinct genetic events involving different oncogenes play a part in the aetiology of neuroblastoma. One event would be the presently uncharacterized change that activates an oncogene related to the *ras* genes. The other event would be the amplification of a presently unidentified oncogene, providing additional template for transcription and increased levels of the gene product. The two changes have not yet been detected in the same cell line or tumour.

Karyotypic evidence of gene amplification has been found in a variety of human tumours^{4,10,11,20-22}, and recent evidence indicates that cellular oncogenes are included in at least some of these amplifications^{6,7,16,23}. Here we have demonstrated that numerous cell lines derived from neuroblastomas share an amplified domain of DNA and that the same domain has been amplified in a neuroblastoma isolated directly from a patient prior to chemotherapy. Our results suggest that amplification of an as yet unidentified cellular gene may be instrumental in the aetiology of human neuroblastoma.

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Requirement for an upstream element for optimal transcription of a bacterial tRNA gene

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Bacterial promoters are the sites at the 5' end of each gene that bind RNA polymerase and direct the initiation of transcription. The functional elements of *Escherichia coli* promoters^{1,2} are two highly conserved sequences, each about six nucleotides long, usually centred at sites -10 and -35, +1 being the initiating nucleotide. We have been interested in the structure of promoters of genes that are subject to stringent control, that is whose expression is reduced in conditions of amino acid shortage, such as rRNA and tRNA genes. We have therefore mapped the sequences involved in promoting *in vivo* transcription of a bacterial tRNA^{Tyr} (*tyrT*) gene by fusing the *tyrT* promoter region to a galactokinase (*galK*) gene, and using *in vivo* expression of galactokinase activity to measure promoter strength. We show here that efficient expression from the *tyrT* promoter requires specific sequences upstream of the canonical promoter elements, and we suggest that these sequences constitute an extended promoter structure.

The wild-type *tyrT* promoter was inserted into the *galK* expression vector pKM-1 (K. M. McKenney and M. Rosenberg, personal communication) as a 300 base pair (bp) *EcoRI*-*AvaI* fragment, which carries 248 bp of *tyrT* sequence upstream of the transcription initiation site, to give pTyr2 (Fig. 1a). Deletion mutations of the *tyrT* promoter were generated by cutting pTyr2 at the *Bst*EII site and then digesting with nuclease *Bal*31. This produced a set of deletions which all share a common 5' end (the *EcoRI* site on pKM-1) and which have 3' ends mapping from -76 to -33 (Fig. 1b). An additional deletion was made at -98 by excising the *EcoRI*-*Bst*EII fragment from pTyr2.

The pKM-1 vector carries a terminator (*At*_{R1}) inserted between the promoter cloning sites and the *galK* gene. We found it impossible to clone the wild-type *tyrT* promoter into analogous vectors lacking a terminator, as has been found in this system with other strong promoters such as *leuT*³ and *rrnB* (M. Cashel, personal communication). Presumably this effect is caused by readthrough transcription from the cloned promoter inhibiting plasmid replication. As the presence of a strong promoter can also influence the copy number of plasmid vectors^{4,5}, we analysed the relative levels of plasmid present in each of the *tyrT*-*galK* fusions in the same growth conditions

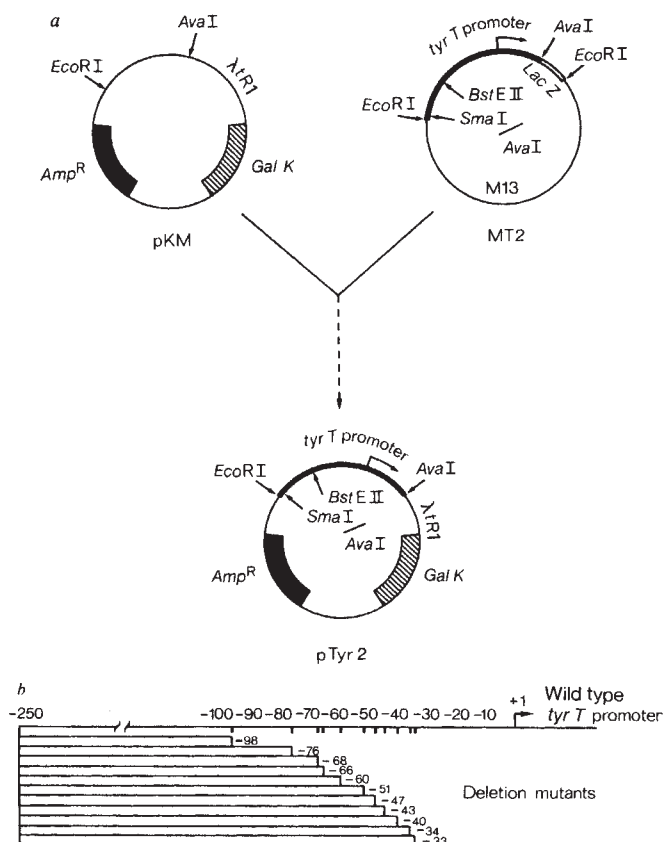


Fig. 1 *a*, Plasmid pTyr2 is a fusion of the wild-type *tyrT* promoter to the *galK* gene of vector pKM-1. It was constructed in two steps using the 500 bp *EcoRI* fragment from MT2, a derivative of M13mp73 with a 500 bp *EcoRI* fragment (originating from pMLB1048, M. Berman, personal communication) inserted that carries the *tyrT* promoter joined to the *lacZ* gene at the *AvaI* site (A.I.L., unpublished). First, the entire *EcoRI* fragment from MT2 was cloned into the *EcoRI* site of pKM-1. This plasmid was then digested with *AvaI* and the resulting three fragments were separated on a 1.4% agarose gel. The large, vector fragment and the small, promoter-containing fragment were purified from the gel and ligated together to generate pTyr2. *b*, The end points of the *tyrT* promoter deletion mutants, which were generated as follows. Plasmid pTyr2 was cut with *BstEII* and the linear plasmid DNA purified over a small DE52 column. The rate of *Bal31* digestion was calibrated using standard assay conditions¹⁰ and then ~3 µg of purified linear DNA were incubated with 3 units of *Bal31* at 30 °C for 3½ min to generate deletions of ~30–60 bp. *Bal31*-cut DNA was then phenol extracted, ethanol precipitated, treated with the Klenow fragment of DNA polymerase and ligated to *EcoRI* linkers. After recutting with *EcoRI* the linear plasmid was purified through a 1% agarose gel, religated and used to transform *E. coli* CB1. The resulting deletion mutants were screened by restriction mapping, and the end points determined by sizing on denaturing polyacrylamide gels¹¹. In the case of Δ-40 the structure was confirmed by subcloning the region into M13mp8 as a *HincII*–*AvaI* fragment and directly sequencing across the breakpoint. Deletion Δ-98 was made by cutting pTyr2 with *BstEII*, repairing the ends with Klenow fragment and ligating on *EcoRI* linkers. Following recutting with *EcoRI* the DNA was run on a 1% agarose gel and the large plasmid fragment isolated, religated and used to transform *E. coli* CB1. Standard conditions were used for ligation, repair, transformation, and so on¹⁰.

used for measuring GalK activities⁵. Table 1 shows the relative copy number of each plasmid together with its measured GalK activity and the value corrected according to plasmid concentration. It can be seen that the actual variation in copy number is quite modest, being at most a twofold effect. However, we do find that the plasmids displaying highest amounts of GalK activity are present in the lowest copy number, supporting the observation that there is an inverse correlation between promoter strength and plasmid levels in the *galK* expression vector

Table 1 Galactokinase expression from wild-type and derivative *tyrT* promoters

Plasmid	GalK activity	Copy no. correction	Corrected GalK activity
pTyr1	135	2.0	270
Δ-98	127	1.9	241
Δ-76	64	1.3	83
Δ-68	60	1.8	108
Δ-66	59	1.3	77
Δ-60	45	1.2	54
Δ-51	30	1.0	30
Δ-47	35	1.7	60
Δ-43	35	1.2	42
Δ-40	21	1.1	23
Δ-34	0	1.2	0
Δ-33	0	1.4	0
pKMSSU1	90	—	—
pKMSSU1 + 1.1 kb fragment, orientation A	60	—	—
pKMSSU1 + 1.1 kb fragment, orientation B	58	—	—

GalK activities were determined as previously described⁹ and are expressed as nmoles galactose phosphorylated per min per ml cells at A_{550} . For each plasmid a minimum of two separate extracts were prepared and assayed in duplicate. The *E. coli* host strain used throughout was CB1, a C600 derivative which is *galK*[−], *recA*[−] and *Tc*^R (A.I.L., unpublished). Plasmid copy number was analysed by the method of Stueber and Bujard⁵.

system (C. W. Adams and G. W. Hatfield, personal communication).

The level of GalK activity produced by the wild-type *tyrT* promoter is very high, approximately 10-fold higher than with the *lacUV5* promoter in similar conditions (data not shown). Fusion of other stable RNA (tRNA and rRNA) promoters to *galK* vectors^{3,4} has shown similar high levels of expression, and the *tyrT* result is consistent with stable RNA promoters being the most active class of chromosomal promoters in *E. coli*.

The graph shown in Fig. 2 presents the corrected GalK activities of the *tyrT* deletions plotted against deletion endpoint. It can be seen that deletions of *tyrT* sequences 5' of position −98 have little or no effect on the efficiency of the promoter. It is clear, however, that larger deletions display a marked reduction in promoter activity, with a deletion to −40 (which still leaves intact the conventional promoter elements) expressing at less than 10% the level of pTyr2 and deletions extending to −34 and −33 producing no GalK activity at all, which confirms that an intact −35 element is essential for *tyrT* promoter function. However, as we find that pTyr2 gives ~10-fold more GalK activity than the *lacUV5* promoter it would appear that deletions that leave the normal promoter region intact can still function at a comparable level with other *E. coli* promoters. The upstream region may therefore constitute a means of increasing transcription from a promoter consisting of regular −10 and −35 consensus sequences.

An alternative, trivial explanation for the phenotypes of the deletion mutants is that there is an inhibitory effect of the vector sequences that are being juxtaposed beside the promoter. As a control against the possible poisonous effect of incoming vector sequences a 1.1 kilobase (kb) fragment of human DNA was inserted into the *EcoRI* site (the deletion breakpoint) of several plasmids, and the effect upon *galK* expression measured (data not shown). In each case insertion of the human *EcoRI* fragment failed to restore wild-type promoter activity and usually resulted in a small decrease from the previous level. We therefore consider it extremely unlikely that the reduced promoter activity of the deletion mutants is caused simply by the proximity of poison sequences.

The sensitivity of the *tyrT* promoter to sequence alterations upstream of the −10 and −35 elements was further demonstrated by analysis of the synthetic *tyrT* gene (*SSU1*) cloned

Fig. 2 Galactokinase activities are shown corrected for variation in plasmid levels. The error limits indicate the accuracy of this correction factor, and are derived by assuming that deletions Δ -33 and Δ -34 should have the same copy number. Hatched boxes indicate the conventional promoter elements.

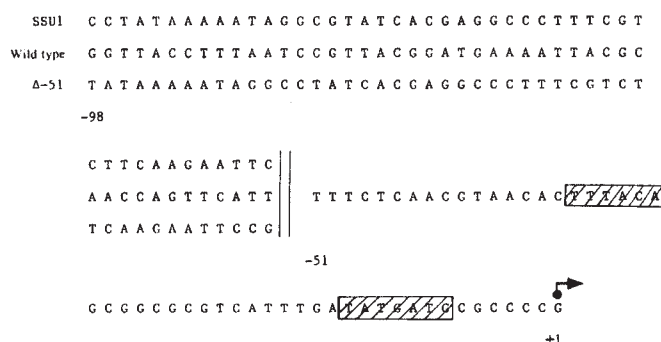
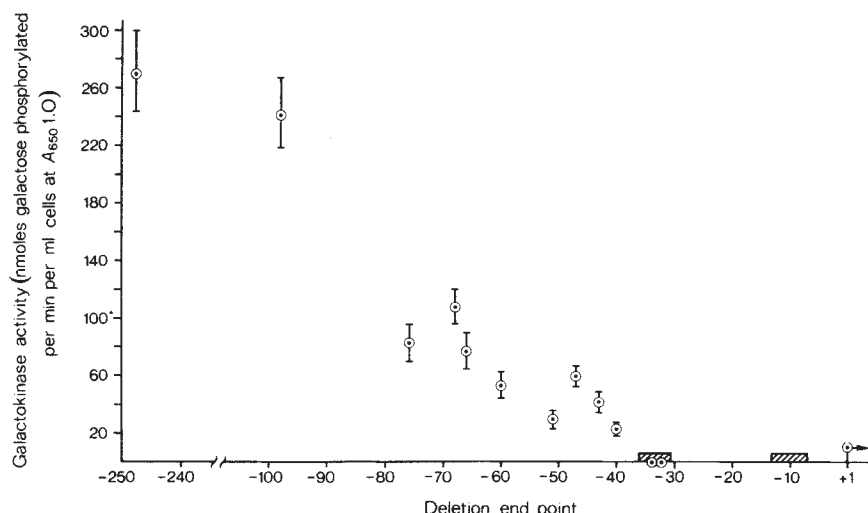


Fig. 3 DNA sequence of the wild-type *tyrT* promoter¹² and mutants Δ -51 and SSU1. The transcription initiation site is shown as position +1 and the conventional promoter elements are shown enclosed by hatched boxes.

by Dunn *et al.*⁶ As shown in Fig. 3, SSU1 carries 51 bp of wild-type upstream sequence followed by an *EcoRI* site, and it was cloned into pKM-1 as an *EcoRI*-*AvaI* fragment analogous to the constructs described in Fig. 1. This plasmid, pKMSSU1, differs from the *Bal*31 mutant Δ -51 only in the sequence of the *EcoRI* linker at the deletion breakpoint and is thus equivalent to a deletion from Δ -51 of the dinucleotide C-G at position -52/53. The GalK activity of pKMSSU1 and derivatives carrying the 1.1 kb fragment of human DNA in both orientations was measured, and the results are shown in Table 1. While still reduced from the wild-type level, pKMSSU1 clearly expresses more efficiently than Δ -51, despite having the same *tyrT* sequence downstream of the deletion breakpoint, and the same vector sequence upstream. Insertion of the human DNA fragment at the deletion breakpoint of pKMSSU1 produces a further decrease in GalK activity; a similar decrease was also observed when the fragment was inserted into the other *tyrT*-*galK* fusion plasmids and it probably results from a lowered copy number of plasmids harbouring the human DNA fragment. However, since the level of GalK activity obtained after insertion of the 1.1 kb fragment into pKMSSU1 is still markedly higher than that of Δ -51, and is identical with the fragment present in either orientation, we think that the precise sequence adjacent to the deletion breakpoint is responsible for the phenotype of pKMSSU1 and not sequences further upstream. (Inspection of the sequences present in the region upstream of -57 reveals no significant homology between wild-type *tyrT* and pKMSSU1 that is also conserved after insertion of the 1.1 kb fragment.) As we do not know the mechanism by which the upstream region stimulates transcription of the wild-type *tyrT* promoter we cannot give an explanation for the difference in promoter activity observed between mutants Δ -51 and pKMSSU1. The result does, however, strengthen the

observation that sequence alterations upstream from the conventional promoter elements can have a strong effect on transcription of the *tyrT* gene. We also note that since pKMSSU1 expresses more GalK activity than Δ -51, while bringing the same vector sequences two bases closer to the promoter, this argues against the incoming vector DNA acting as a general poisoner of promoter activity.

We have shown that DNA sequences mapping between positions -40 and -98 are required for optimal transcription of the *E. coli tyrT* gene. Previous analyses of *tyrT* transcripts, both *in vivo*⁷ and *in vitro*⁸, locate the transcription initiation site at +1 as shown in Fig. 3, and do not reveal transcription starts further upstream. The region of DNA between -40 and -98 does not contain promoter-like sequences orientated towards the *tyrT* gene and does not generate transcripts *in vitro* (A. I. Lamond and A. A. Travers, unpublished observations). Therefore we consider it extremely unlikely that the upstream region increases transcription of the *tyrT* gene by virtue of it containing another independent promoter, although it cannot formally be excluded that some type of promoter with an unusual structure is present which is active *in vivo* but not in the purified *in vitro* system. Therefore, since the required upstream region appears not to dictate ancillary transcription starts, we suggest that it acts to increase the efficiency of the -10 and -35 promoter elements, either by interacting with RNA polymerase itself or by binding some unidentified transcription factor. The main conclusion we have drawn from this work is that the *E. coli tyrT* gene may possess a novel type of promoter structure that allows highly efficient expression. We see the major implication of this as being that the *tyrT* promoter structure could be common to many other genes that are expressed at high levels, such as other tRNA genes, and we speculate on the possible involvement of the upstream element as a regulatory determinant of stable RNA production *in vivo*.

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Predicting disasters

Peter D. Marshall

Earthquake Forecasting and Warning.

By Tsuneji Rikitake.

D. Reidel: 1982. Pp.400. Dfl 120, \$45.

EARTHQUAKE prediction, once the domain of astrologers, soothsayers and an elderly lady in Edinburgh, emerged some 15 years ago as an important science with potentially alarming social consequences. Evidence for this is provided by Prof Rikitake in his latest book *Earthquake Forecasting and Warning*, the third volume in a series *Development in Earth and Planetary Sciences* which is edited by Rikitake. This new volume is effectively a state of the art report on the prediction programme and in many ways is an update of his earlier book on earthquake prediction.

The prediction programme has become a growth industry for geoscientists in several nations, including the USA, USSR, China and for obvious reasons Japan. The international co-operation in this socially important programme is impressive: US scientists have been allowed in to the USSR to monitor premonitory microearthquakes, thought by many to be important precursors to larger and more hazardous earthquakes but they are not yet allowed in to record seismic activities as part of an arms control agreement. China has welcomed and encouraged exchanges of scientists from many countries to study prediction methods and demonstrate their own prediction strategy.

Although there is much exchange of the observational evidence the success of any prediction programme must ultimately depend on the geoscientists ability to understand and exploit any geophysical, geochemical and biological anomalies which may precede a hazardous earthquake. The Chinese would also include an understanding of the macroscopic anomalies: the premonitory anomalies observed by the eye, nose and ear which they have used so successfully to predict earthquakes. There exists at present no definitive model to explain the observed premonitory phenomena. The dilatancy model still remains a popular explanation but only of the precursors associated with changes in crustal stress. Many large earthquakes occur for which there are no phenomena; clearly the science still has a long way to go.

Scientists not involved in the prediction programme may be unaware of the complexity of making a prediction public. Given a set of

premonitory observations the geoscientist faces his most difficult task: the assessment of the probability of when and where a hazardous earthquake will occur. The strategy of prediction is equally complex and will surely vary from country to country. A final decision may be too difficult to make; it is a view held by many geoscientists that there still remains an acute shortage of positive data on which to base a prediction and that what little exists is of questionable validity. If this is so it will take a brave scientist to alert his government of an impending hazardous earthquake.

Given an announced warning of an earthquake, how will the public react? The warning could lead to a chaotic exodus from the threatened area. Imagine the consequences of trying to evacuate San Francisco in a hurry! Paradoxically, as the predictions become more and more successful the panic level could increase dramatically. In California it is claimed that 49 deaths could be attributed directly to the emergency evacuation following the successful prediction of a Bay Area earthquake in February 1979. Strangely no nation has a substantial programme to investigate the reaction of the public to earthquake warnings. Surely there is scope here

for research by social scientists. At least, Japan and the USA have introduced legislation to control public response to a warning but only time will show how effective this will be.

At present the success rate of earthquake warnings is not high. The most successful short-term (hours-days) prediction is that of China in which the Haicheng earthquake of 1975 was predicted with sufficient accuracy to alert the high risk region. The shock, fortuitously perhaps, was preceded by a plethora of premonitory phenomena including anomalous animal behaviour. Whilst it is recognized that a real scientific basis existed for the prediction, much use was made of amateur observer participation: a feature of the Chinese programme not seriously exploited in the West.

To date most of the research has centered on the acquisition of premonitory data, more effort is needed on the basic research to produce a fundamental understanding of the physical processes of premonitory phenomena. Plate tectonics may explain why and where some earthquakes occur but researchers should not be constrained by a theory in which a fault is stressed by a force applied some thousands of miles away and transmitted horizontally through plates; vertically applied stress may also play an important role in triggering earthquakes. Perhaps more effort should be focused on making a population safe from the direct and indirect effects of hazardous earthquakes.

Anyone concerned, or with an interest in the problems and consequences of earthquake prediction or warning will find Rikitake's book most readable and an excellent reference book with an extensive collection of observed data and references on all aspects of the prediction programme. It brings the reader up to date with recent progress in Japan, China and the USA placing emphasis not only on the collection of data but on the strategy of prediction in the different countries. At times the book which provides a very comprehensive treatment of the subject reads like a catalogue, which in places it is, but at other times it is quite thought-provoking. Unfortunately by refraining from a critical evaluation of the many premonitory phenomena Rikitake does not give us the benefit of his wide experience in earthquake prediction. But will we soon be able to make short-term predictions of hazardous earthquakes? Rikitake and many other researchers are quite optimistic about the prospect, but I wonder? □

IMAGE
UNAVAILABLE
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REASONS

Do reliable phenomena precede an earthquake? Chinese officials inspect damage caused by an earthquake in Jiangsu Province, E. China, in 1979.

Xinhua

Peter D Marshall is a Principal Scientific Officer in the Seismology Division of the UK Ministry of Defence, Aldermaston.

Of stars and planets

David W. Hughes

100 Billion Suns: The Birth, Life and Death of the Stars.

By Rudolf Kippenhahn.

Basic Books, New York/Weidenfeld and Nicolson: 1983. Pp.264 \$25, £15.

The New Solar System (2nd Edition).

Edited by J. Kelly Beatty, Brian

O'Leary and Andrew Chaikin.

Cambridge University Press: 1983.

Pp.240. £12.50, \$24.95.

EJNAR Hertzsprung and Henry Norris Russell provided one of the most fruitful avenues of astrophysical display when they independently plotted the colours (or spectra) of stars as abscissae against their absolute magnitudes (or luminosities) as ordinate. This H-R diagram has become the backbone of all discussions of stellar evolution, and its main sequence and giant branch are the temporary dwelling places of the large majority of stars. The tortuous path of a star around this diagram illustrates graphically the change in size, surface temperature and energy generation process that occur to a star of a specific mass as it evolves.

Many of the most fascinating problems of astrophysics are enshrined in this evolution. Why do some clouds collapse to form stars, when and how do stars change nuclear fuels from hydrogen to helium, what is the role of mass loss in evolution, what is the dividing line between white dwarfs and neutron stars, pulsars and X-ray sources, why do some stars become Cepheids?

Kippenhahn has spent the large majority of his research career investigating stellar evolution using sophisticated computer models to represent the physical characteristics and nuclear processes in the interior of stars. To him stars are not merely phenomena inviting our awestruck admiration. They are objects of our universe and as such are subject to the law of physics. Chapter by chapter he unravels their intricacies. At the end the reader is left with a vivid understanding of how stars work.

The reader is continuously aware that the author has had first hand experience of the subject. It is also obvious that many of the astronomers mentioned in the book are the personal friends and acquaintances of Rudolph Kippenhahn and his anecdotes and insights into the workings of this branch of astrophysics are fascinating. The illustrations and figures are excellent. The non-mathematical exposition is clear and precise and this book is an admirable introduction to the difficult subject of stellar evolution accurately aimed at the general reader. The book under review is a translation of the German edition which was published in 1980 (Piper & Co., Munich).

Only fifteen months separate the first and second edition of *The New Solar System* but fifteen months is a long time in planetology. Voyager 2 has rushed past Saturn and two Soviet spacecraft have plummeted through the clouds to land on the surface of Venus. The 'average scientist' has been to two or three conferences in that time and written half a dozen or more research papers. There is plenty to update.

The first edition contained twenty chapters each of about ten pages. The authors were American, the list reading like a 'Who's Who' of solar system experts. Typical examples are Van Allen on magnetospheres and the interplanetary medium, Shoemaker on cratering, Masursky on Mars, Chapman on asteroids, Burns on rings, Pollack on Titan and Wood on meteorites. No change in chapter size has been allowed in the new edition. The only major difference between the two is the append-

ing of a 14 page map section. Just imagine the U.S. Geological Survey map of Mercury miniaturized to fit into an area 17 x 23 cm and you quickly realize that people without a handy magnifying glass will find these maps of very limited use. Most of the lettering is unreadable.

However, people will not buy this book for the maps but they *should* buy it, because it is well written, full of superb photographs, beautiful artists impressions and excellent figures. It is a first rate introduction to the solar system and a book against which popular astronomy books of the future will be measured. Too little has changed to make it imperative for owners of the first edition to buy the new one. But if you haven't got edition one, and have the slightest glimmer of an interest in the solar system, get this book. □

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Making waves

F.A. Dahlen

Seismic Wave Propagation in Stratified Media.

By B.L.N. Kennett.

Cambridge University Press: 1983.

Pp.342. £30. \$59.50.

IT HAS been said there are two good times to write a specialized book on a scientific topic. One is when a new field is just beginning to open up and a well-written treatise can pave the way for future research; the other is when a field is mature and fully developed, ripe for its elegance and unity to be revealed.

Kennett's account of the theory of seismic wave propagation in stratified media is a superb example of a book in the second category. Spurred by a number of diverse impetuses, the construction of synthetic seismograms to aid in structural and seismic source studies has progressed rapidly during the past two decades. Kennett has summarized this progress just at a time when the methods have become fairly well established and the attention of many seismologists is turning away from stratified media towards the investigation of lateral heterogeneity on a variety of scales.

When first asked if I would review this book, I considered my already crowded schedule and thought I should decline in favour of a more knowledgeable colleague. I'm glad I didn't because I learned a great deal by taking the time to read it slowly and carefully. Kennett begins by setting up the matrix framework appropriate to stratified media, following this with a brief discussion of seismic point sources.

The main unifying theme is introduced next in two long (and occasionally difficult) chapters on the reflection and transmission properties of the stratification. The subsequent chapters describe both phase and slowness methods for constructing complete theoretical seismograms, as well as less-expensive systematic approximations to the full response useful in many applications. The emphasis throughout is on planar stratification, with occasional paragraphs or brief sections on the extension to the spherical case. All the important recent developments due to Chapman, Fuchs, Kennett himself, Müller, Richards, Woodhouse and others are clearly described here and compared in the context of the reflection matrix approach.

The usual high production standards of the Cambridge *Monographs on Mechanics and Applied Mathematics* have been maintained, although I did detect a fair number of typographical errors and omissions. Kennett is to be congratulated in particular for devising a consistent and reasonably self-explanatory notation, but a glossary would have been helpful — I frequently found myself thumbing back through to remember the difference between $\mathbf{R}^{\downarrow}_{\downarrow}$ and $\mathbf{R}^{\downarrow}_{\downarrow}$, or the meaning of $\tilde{\mathbf{W}}$.

In summary, this is a model mathematical treatise, written with authority by an expert but accessible with diligence to all seismologists. It would be an excellent basis for a topical seminar on advanced seismology. Together with Aki and Richards's *Quantitative Seismology* (W.H. Freeman, 1980), the book certainly should be mandatory reading for theoretical seismologists for some time to come. □

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And in the beginning

Stephen Moorbath

Geological Evolution of the Earth during the Precambrian.

By Lazarus J. Salop. Translated by V.P. Grudina.

Springer-Verlag: 1983. Pp.459. DM148, \$65.80.

AN INFLUENTIAL sector of the Soviet geological establishment has always rejected the concepts of, and evidence for, sea-floor spreading, continental drift and plate tectonics. The result is that modern views in the USSR concerning the geological evolution of continents, oceans, crust and mantle, follow on closely from the days immediately preceding the great revolution in global tectonics of the early 1960s which so heavily influenced most of the rest of the geological world (including, I suspect, many younger workers in the USSR).

Exactly the same situation prevails in the book under review. It reads almost as if most of the great advances of recent years in geophysics, geochemistry, geochronology, isotope geology, petrogenesis — not to mention field geology — which have so shaped our thinking about Precambrian evolution, had never actually happened. What limited reference there is to what most workers would regard as essential modern background work is mostly either misunderstood or discredited.

Here we still find classical geosynclinal theory in full flower; division of Earth history into numerous cycles, megacycles, orogenies of different "orders", all with impressively exotic names; preservation of the major ocean basins, floored by ancient, subsided, continental crust mainly composed of granulite gneisses, right through from Precambrian times; ubiquitous granitization of supracrustal rocks on every continent during the Katarachaeon (c. 4,050–3,750 Myr) leading to a world-wide, primordial granulite crust; production of gneiss domes by astrobleme impacts; and so on. Palaeomagnetic evidence is taken to support polar wandering, whilst the overall tectonic evolution of the continents is explained by a pulsation hypothesis — alternate periods of expansion and compression within the Earth being seen against a background of relatively small but increasing expansion of the planet, reaching its maximum value at the end of the Phanerozoic.

In the well-studied area of early Precambrian rocks in West Greenland, which I happen to know well, the author invents a "Godthaabian orogeny", dated at 4,060–4,000 Myr and divided into Slyudyankan, Sutamian, Fedorovian, Ungran and Iyengran sub-units. In truth, the oldest rocks mapped in the field in this area are the Isua supracrustals, dated at close on 3,800 Myr, which carry no isotopic imprint whatever

of significantly older crustal residence.

And so I could go on. Much of what is postulated in this curious, scholarly, perverse, obstinate text deserves to apply to some planet or other. But does it apply to *this* one? I think not! Nonetheless, the book abounds in detailed, useful descriptions of many areas of Precambrian rocks from all continents, accompanied by sketch maps and (in my view) highly speculative, world-wide correlation diagrams.

I shall occasionally take my review copy off one of my more inaccessible bookshelves to drool over the many mouth-watering descriptions and maps of Precambrian rock formations of the USSR. My hope will be that sooner rather than later the acceptance of modern techniques, data and concepts will lead to a more realistic appraisal of these rocks, and of the Precambrian as a whole. □

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Demand for power

Jerrold H. Krenz

World Energy Supply: Resources. Technologies. Perspectives.

By Manfred Grathwohl.

Walter de Gruyter: 1982. Pp.450. DM120, \$54.

BEFORE 1973, the year of the Arab oil embargo, energy supplies were generally seen as being virtually unlimited. The idea that consumption in the industrialized nations could continue to increase well into the twenty-first century coloured all projections of energy usage. Yet not only are fuel resources finite but it appears that new technologies are unlikely to produce gluts of low-cost energy supplies. Realistic appraisals of resources and technological limitations are necessary if we are to form a credible image of the future.

In *World Energy Supplies*, Manfred Grathwohl provides this much-needed appraisal. His coverage is thorough and detailed — the book is intended for the serious reader. The collection of references alone (a total of 784) should be of particular interest to the many students of energy.

The text is divided into four main parts, the first of which deals with population, energy consumption and economic activity. In addition to world data, regional and national comparisons are included along with projections of future demand based primarily on a study by the World Energy Conference (1978). Projections of demand were revised downward very substantially during the 1970s; as Grathwohl puts it "energy conservation potentials have been repeatedly underestimated".

A discussion of energy reserves, primarily fossil and uranium fuels, and

solar insolation follows. The coverage here is extremely useful in establishing a geographical perspective of reserves and their relationship to consuming regions.

The third part provides a lucid description of energy conversion technologies for producing "secondary energy carriers", again on a global basis. Both water-cooled and advanced nuclear reactors are discussed, fusion reactions and experiments are described, thermal and photovoltaic solar collectors are appraised, and indirect solar energy systems are covered.

The next chapter, "Environmental Impacts and Safety Problems", is considerably more detailed than similar accounts found in most energy texts. All too frequently these aspects are considered to be of peripheral importance only. Grathwohl affords such constraints — the build-up of atmospheric carbon dioxide, for example — the attention they deserve; while difficult to ascertain, environmental limitations may prove to be more important than resource limitations. The treatment of the entire fission fuel-cycle, accident possibilities and the diversion of fissionable materials for weapons is particularly well done. Proposed disposal schemes for high-level wastes are also discussed, the author recognizing that waste disposal will require a much greater effort than at present. Unfortunately, he offers no suggestions on how to get things moving — that there may be "no recognizable technical or scientific reasons why it [permanent storage] should not be practical" is a worn phrase. It is time to initiate an effort commensurate with the seriousness of the problem.

As Grathwohl repeatedly stresses, the adequacy of energy supplies, especially given the long lead-time necessary for new technologies, depends on future demand. But demand is poorly understood. We lack a body of literature from which to prepare a treatment of energy utilization as extensive as that which Grathwohl offers on energy supplies. Basic research and the accumulation of data on energy usage has hardly begun. Does the myth of unlimited resources still cloud our perception? Would a serious attempt to understand better energy usage be tantamount to acknowledging that our resources are limited and thus constitute too great a threat to one of our cherished myths? While *World Energy Supply* does not quite answer these questions, it does provide a valuable overview of energy problems; for that Grathwohl is to be thanked. □

Jerrold H. Krenz is on the Electrical Engineering Faculty at the University of Colorado at Boulder. He is author of *Energy: From Opulence to Sufficiency (Praeger/Hemisphere, 1980)* and *Energy: Conversion and Utilization (Allyn and Bacon, 1976)*.

● The German edition of *World Energy Supply: Resources. Technologies. Perspectives*. Vol 2 by M. Grathwohl has just been published by Walter de Gruyter; price DM136.

Magnetic attractions

B.A. Hobbs

Introduction to Geomagnetism.

By W.D. Parkinson.

Scottish Academic Press/Elsevier

Scientific: 1983. Pp.433. £27.50, \$49.50.

Geo-Electromagnetism.

By James R. Wait.

Academic: 1982. Pp.280. £22.60, \$34.

SEISMOLOGY, gravity, heat flow and geodynamics all contribute to an understanding of the Earth and its internal structure but are mainly restricted to the study of its present state whereas the study of geomagnetism, which includes electric as well as magnetic fields, addresses questions relating both to the Earth's history and to its present dynamic atmospheric and magnetospheric environment.

Geomagnetism was fundamental in developing the ideas of plate tectonics and continental drift and alone enables the positions of ancient continents to be reconstructed. It raises questions, and provides some of the answers, as to processes within the Earth's core, both past and present. At the other extreme, magnetic and electromagnetic techniques can be used to study the fine structure within the Earth's crust and they have been exploited in hydrocarbon and mineral exploration. Outside the Earth, complex interactions take place between plasmas, magnetic fields and the Earth's atmosphere, electric currents flowing tens of Earth radii away influence magnetic fields measured at the surface of the Earth. Indeed, from these surface measurements the ionosphere and magnetosphere were first postulated, later to be confirmed by rocket and satellite measurements.

To present a unifying treatment of these diverse topics is a Herculean task. *Introduction to Geomagnetism* has achieved that task, and even more. The similarity in title of the other book examined, *Geo-Electromagnetism* belies the difference in subject matter, aims and approach. Although both books touch on topics such as Magnetotelluric Theory, the latter concentrates on theory underlying geomagnetic (mainly electrical) prospecting methods used for detailing near surface structure.

The extraction of information from geomagnetic data requires an understanding of the physical principles of magnetism and the measurement of relevant magnetic fields. The geomagnetic field is monitored more or less continuously by some 120 permanent magnetic observatories, and spasmodically by local surveys, marine and airborne surveys, by satellite and space probes and by measuring the magnetization of rock samples.

In tackling this, Parkinson's logical

approach is to divide the geomagnetic field into four main classifications, the main field, the local field, external fields and internal fields. Within each classification, the necessary theory is developed; magnetohydrodynamics for a discussion of the main field, rock magnetism for the local field, plasma conductivities for external fields and electromagnetic induction for internal fields. Modern theories, explanations and results predominate but each chapter is flavoured with historical developments. A fascinating section on the history of geomagnetism is included in its own right. The whole range of geomagnetism is treated in a refreshingly logical manner by classifications and sub-classifications.

Parkinson has the rare combination of scientific insight and the ability to write with clarity and interest. His name is firmly established in geomagnetism through the terminology of Parkinson planes, originally proposed for describing induction effects at ocean-continent boundaries. Having dedicated his life to geomagnetism (as did his father), Parkinson is well qualified to write an *Introduction to Geomagnetism* — the result is an excellently prepared and produced book which should be regarded as essential

reading for undergraduates and postgraduates in geophysics.

Whereas Parkinson is concerned exclusively with natural phenomena, in *Geo-Electromagnetism*, Wait concentrates on the use of induced magnetic and electric fields arising from artificial sources for exploration. In all cases the source gives rise to local electric and magnetic fields which are influenced by the conductivity, or a related parameter, of the underlying media. These local fields can be detected and the measured response compared to the computed response of various ideal structures in an attempt to determine compatible models. Wait has been at the forefront of this exploration method for many years and has been concerned both with theoretical developments and applications, but restricts himself here to the theory underlying the various source and detector types and configurations. It is a useful compendium of formulae relating to methods of electrical prospecting and many examples of computed responses from simple ideal structures are included in the book. □

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Lean facts

Harold Baum

Concise Encyclopedia of Biochemistry.

Translated, revised and enlarged by

Mary Brewer and Thomas Scott.

Walter de Gruyter: 1983. Pp.518.

DM59, \$29.95.

WE biochemists work on our papers, and grant applications, read dissertations and mark essays at home and on bus, train or plane. (And students, of course, also read their notes and write their essays equally peripatetically.) Hence there would seem to be a place for a concise "dictionary", provided that it meets some very demanding criteria.

It must be small and light enough to fit into the brief-case and to use on a crowded train. The topics must be easy to locate. The print must not be too small nor the pages too flimsy, and yet the content must be comprehensive. These constraints require succinct but authoritative entries and careful cross-referencing.

The Concise Encyclopedia of Biochemistry (translated and revised from the second edition of the Brockhaus ABC Biochemie) represents a creditable attempt to meet such a need. I invited several colleagues to look up items that interested them and usually they found the entries to be adequate and accurate. Inevitably, oversimplification of complex topics has led to error (e.g. the subunit structure and activities of acetylcholinesterase) but the con-

sensus was that the book is very sound in what it presents, if irritating in what it omits (e.g. no entry for alloxan or streptozotocin).

What caused more annoyance was the location of entries — nothing under "Aminotransferases", but a good entry under "Transaminases, aminotransferases"; nothing under "ATPase", nor (following cross-reference to "Adenosine phosphates") under "Adenosine triphosphatase"; nothing under "Sodium", but "Na⁺K⁺-ATPase" cross-referred me to "Transport" where I found what I was looking for!

Nevertheless, once you get used to the system, and provided that you have a good idea of what you are looking for, it is not difficult to find your way around. Also, the cross-referencing realizes the bonus of all good dictionaries — you find yourself browsing. If I hadn't been looking for the Na⁺K⁺-ATPase I wouldn't have found the excellent tables summarizing the occurrence and effects of cyclic AMP.

This, then, is not a standard reference work, not an alternative student text, not an essential library book; but it is a useful little book to have around the lab., at home, on the desk or in the brief-case. And if readers write to Drs Scott and Brewer as requested, suggesting new entries, and if those compilers in their turn keep all the entries up to date, the next edition should be better — provided that it stays small. □

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Nature Boston Conference

The companies featured this week will be exhibiting at the Nature conference, to be held in Boston, USA, from 19 to 21 September, 1983.

● Two new ion exchangers — DEAE-sepharose fast flow and CM-sepharose fast flow — have been introduced by Pharmacia Fine Chemicals. The gels are derived from the widely used DEAE- and CM-sepharose CL-6B gels by a modification of the cross-linking. This increases flow rates to 180 cm h^{-1} in "Stack" and Sephamatic production columns.

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● A line of chromosome media and reagents for *in-vitro* diagnostic cytogenic studies is now being offered by Gibco Laboratories. This product line includes not only complete culture media, but also the necessary auxiliary reagents to perform these assays. A chromosome sex-test kit is also available that includes all necessary reagents to perform sex assays.

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● Amicon has introduced a new line of laboratory columns for medium-pressure liquid chromatography with pressure ratings from 45 to 100 p.s.i. ($3\text{--}7 \text{ kg cm}^{-2}$). The new Amicon/Wright columns have a locking adjuster assembly, and precision support nets which are moulded with snap-on rims.

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● Two new digital gauge controls — an ionization model and an ionization/thermocouple model — have been introduced by Varian. Called the 880-1 and 880-2TC, the units offer automatic electronic range changing, LED digital displays for pressure measurement in torrs, millibars or Pascals, and self-diagnostic error code displays. The 880-1 ionization gauge control model operates with both standard and broad-range Bayard-Alpert tubes. When Varian's 564 broad-range ion gauge tube is used, a linear response is attainable from 1×10^{-1} to 4×10^{-10} mbar for argon. The 880-1 is equipped with two ion set-points. In addition to the features of the 880-1, the 880-2 has two independent thermocouple gauge controls and two thermocouple set points.

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● A continuous-flow analysis (CFA) software package called CFAS/3350 has been developed by Hewlett-Packard for the HP 3357 and 3356 laboratory automation systems (LAS). All of the usual wet-chemistry steps are carried out automatically as the sample stream flows through the instrument. CFA instruments include technicon autoanalysers and atomic-absorption spectrophotometers with automatic samplers. CFAS/3350 automates calculation procedures routinely performed in the pharmaceutical, clinical, environmental, chemical and related industries.

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● Gibco Laboratories now supplies a new medium, GCT cell conditioned medium, for use in physiological and chemical studies. GCT conditioned medium is obtained from the growth of giant cell tumour cells and displays colony-stimulating activity.

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● Pharmacia Fine Chemicals have launched a new liquid chromatography controller (LCC) system for automating fast protein liquid chromatography and traditional chromatography.

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Pharmacia's liquid chromatography controller



Gibco Laboratories' GCT medium

● A line of Durapore thermoplastic (TP) cartridges for $0.2 \mu\text{m}$ sterile filtration is now available from Millipore Corporation. The line includes hydrophobic and hydrophilic cartridges for gas and liquid filtration in three lengths and four different O-ring configurations to fit various housings. Durapore TP cartridges have a single-layer, fluorocarbon membrane which offers a greater surface area, higher throughputs and lower pressure drops. This is combined with validated retention and low extractables.

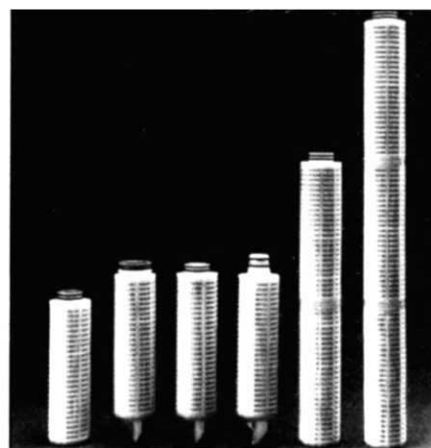
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● Bio-Rad's new Econo-Column calibrators provide a way to determine column bed height and bed volume. The calibrators are self-adhesive, transparent Mylar strips with permanent markings.

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● Human T-cell growth factor, or interleukin-2, is now being marketed by Cellular Products. It is obtained from a pool of at least 100 blood donors, and is tested for long-term growth. The interleukin-2 is available in either phytohaemagglutinin induced, delectinated form, or TPA induced, delectinated, serum-free, protein-free form.

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